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(54) **GENETICALLY MODIFIED PLANTS WHICH SYNTHESIZE A LOW AMYLOSE STARCH WITH INCREASED SWELLING POWER**

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(58) **Field of Classification Search** None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to genetically modified monocotyledonous plant cells and plants whose starch has an apparent amylose content of less than 5% by weight and an increased activity of a protein with the activity of a starch synthase II and an increased activity of a protein with the activity of a glucan, water dikinase. Such plants synthesize starch with an increased hot-water swelling power. Methods and processes for the generation/preparation of these plant cells, plants, starches and flours are likewise subject matter of the present invention.

17 Claims, 2 Drawing Sheets

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Determination of SS2-Activity in transgenic lines

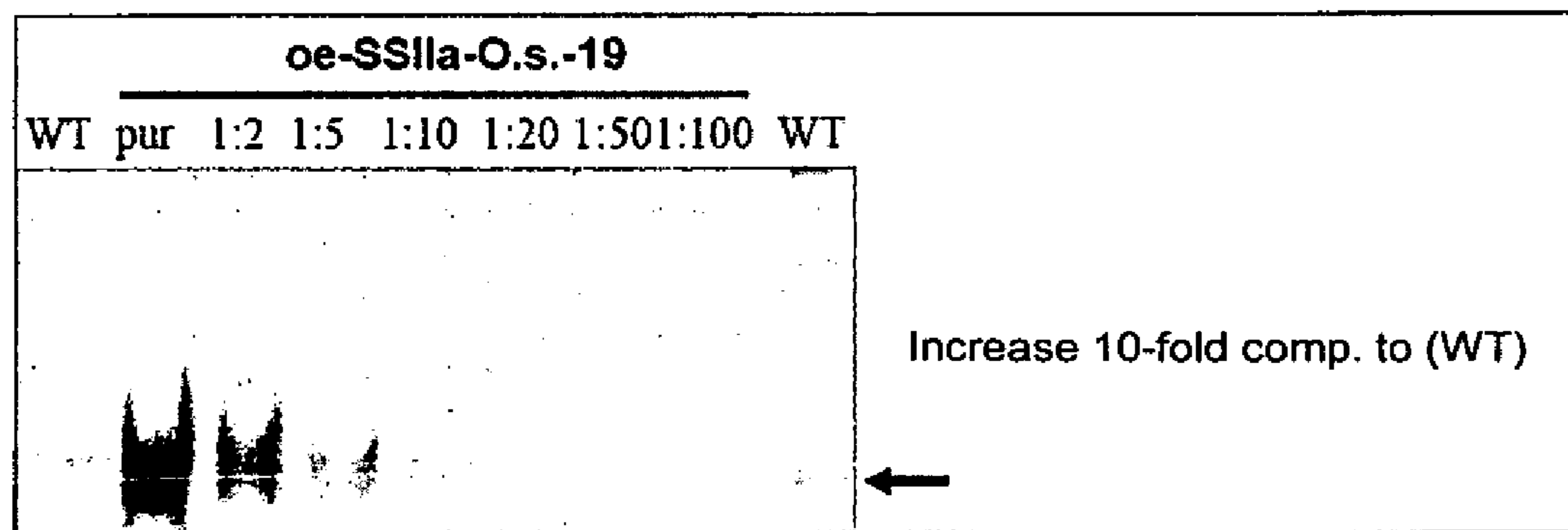
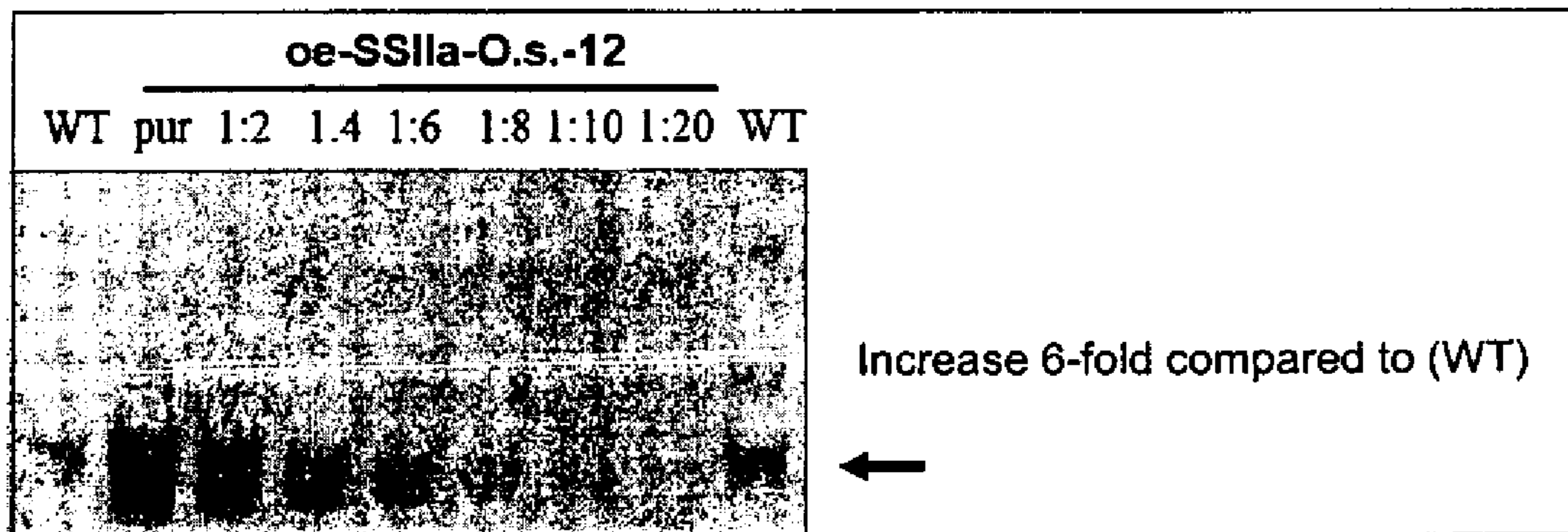
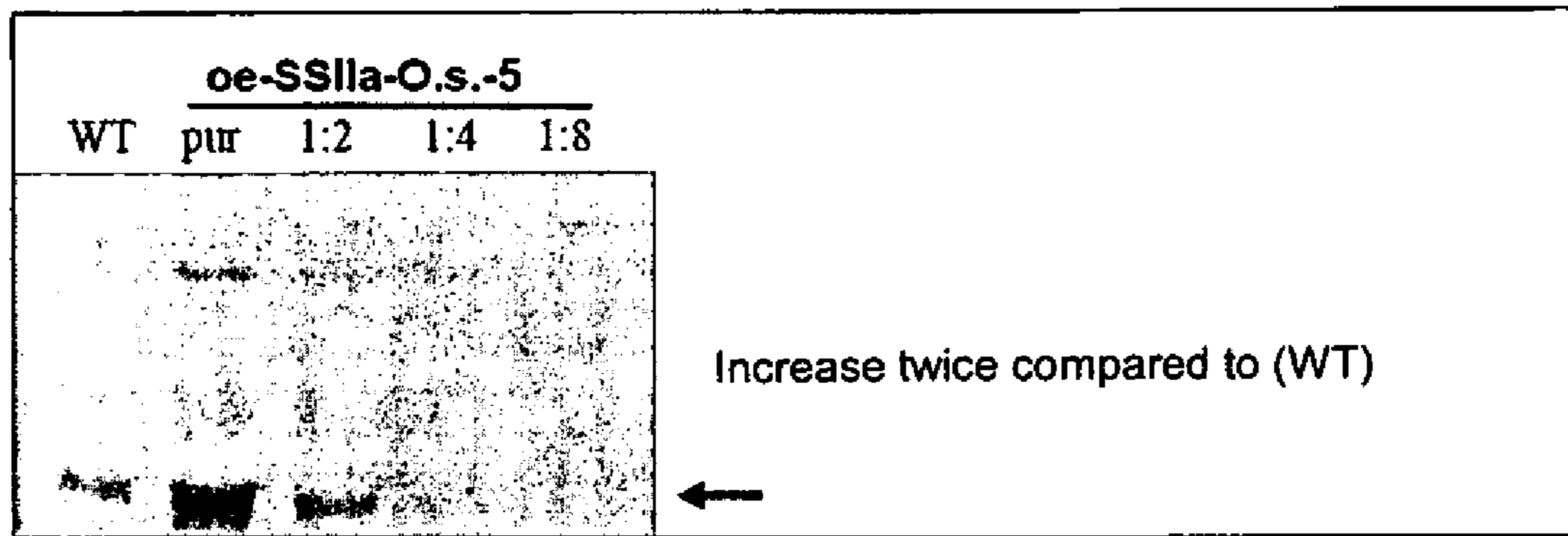


Fig. 1

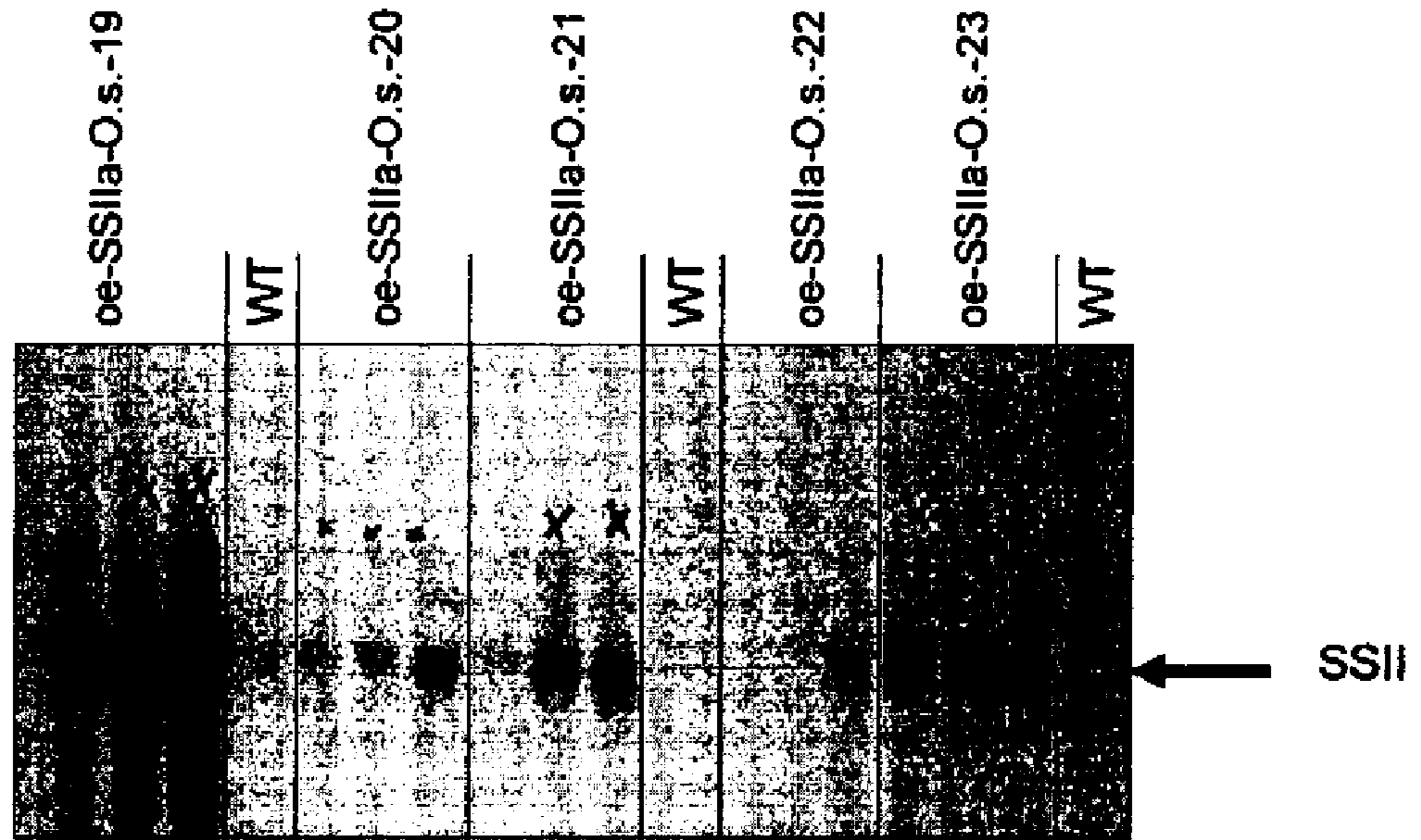


Fig. 2

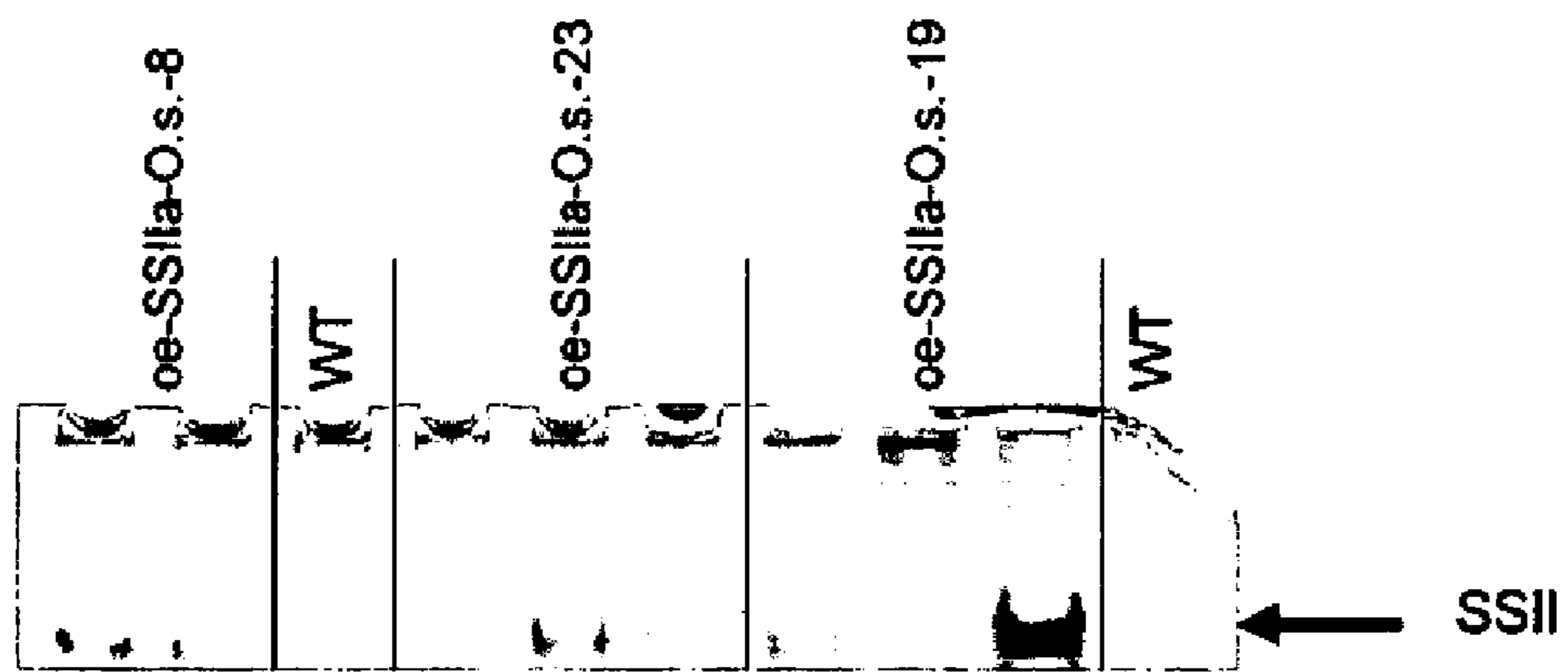


Fig. 3

**GENETICALLY MODIFIED PLANTS WHICH
SYNTHESIZE A LOW AMYLOSE STARCH
WITH INCREASED SWELLING POWER**

CROSS-REFERENCE TO RELATED
APPLICATIONS

This application is the U.S. National Stage filing of International Application No. PCT/EP2008/000614, filed Jan. 23, 2008, which claims priority to European Patent Application No. EP 070 90 009.7, filed Jan. 26, 2007, and U.S. Provisional Application No. 60/898,427, filed Jan. 30, 2007, the disclosures of which are hereby incorporated in their entirety by reference.

The present invention relates to genetically modified monocotyledonous plant cells and plants whose starch has an apparent amylose content of less than 5% by weight and an increased activity of a protein with the activity of a starch synthase II and an increased activity of a protein with the activity of a glucan, water dikinase. Such plants synthesize starch with an increased hot-water swelling power. Methods and processes for the generation/preparation of these plant cells, plants, starches and flours are likewise subject matter of the present invention.

Besides oils, fats and proteins, polysaccharides are the most important renewable resources from plants. Starch, which is one of the most important reserve materials in Higher Plants, plays a central role in the polysaccharides, besides cellulose.

Furthermore, starch is a nutritionally essential component of human and animal food. The structural features of the starch which is present in foodstuffs may have an effect on the functional properties (for example water-binding capacity, swelling power), the nutritional characteristics (for example digestibility, effect of the foodstuff on the glycemic index) or the structural characteristics (for example sliceability, texture, stickiness, processability) of a very wide range of foodstuffs. Food products therefore frequently comprise a starch with specific structural features which bring about the desired characteristics of the foodstuff in question. Also, the starch which is present in the plant tissues may affect the characteristics of foodstuffs which comprise starch-storing plant tissues (for example grains, fruits, flours).

The polysaccharide starch is a polymer made up of chemically uniform units, the glucose molecules. However, it constitutes a highly complex mixture of different forms of molecules which differ with regard to their degree of polymerization, the occurrence of branches of the glucose chains and their chain lengths and which, moreover, may be modified, for example phosphorylated. Starch therefore does not constitute a uniform raw material. In particular, one differentiates between amylose and amylopectin. In typical plants used for industrial starch production or as foodstuffs such as, for example, maize, rice, wheat or potato, amylose accounts for approximately 20%-25% and amylopectin for approximately 70%-80% of the synthesized starch.

The functional, nutritional or structure-imparting characteristics of starch such as, for example, solubility, the retrogradation behavior, the water-binding capacity, the film-forming properties, the viscosity, the gelatination properties, the freeze-thaw stability, the stability to acid, the gel strength, the swelling power, the digestibility, the size of the starch grains of starches are affected, inter alia, by the structural features of the starch, such as the amylose/amylopectin ratio, the molecular weight of the glucose polymers, the side-chain distribution pattern, the ion content, the lipid and protein content and/or the starch grain morphology.

Methods based on plant breeding may be used to modify selected structural characteristics of the starch and therefore functional, nutritional or structure-imparting characteristics of starch in plant cells. However, at present this is only possible for selected structural features of starch (for example amylopectin/amylose content, U.S. Pat. No. 5,300,145). It is not possible currently for example to influence the starch phosphate content in plants by plant breeding methods alone.

An alternative to plant breeding methods is the targeted modification of starch-producing plants by means of recombinant methods. However, a prerequisite for doing so is the identification and characterization of the enzymes involved in starch synthesis and/or starch modification, and their subsequent functional analysis in transgenic plants.

A variety of enzymes which characterize different reactions are involved in the synthesis of starch in plant cells. Starch synthases (EC2.4.1.21, ADP-glucose:1,4-alpha-D-glucan 4-alpha-D-glucosyltransferase) catalyze a polymerization reaction by transferring a glucosyl residue from ADP-glucose to alpha-1,4-glucans, where the glucosyl residue transferred is linked with the alpha-1,4-glucan by generating an alpha-1,4-linkage. Several isoforms of starch synthases have been identified in each of the plants studied to date. Two classes of starch synthases can be distinguished: the granule-bound starch synthases (GBSS) and the soluble starch synthases (in the context of the present invention also abbreviated to "SS"). Granule-bound starch synthases catalyze the synthesis of amylose, while soluble starch synthases are involved in the synthesis of amylopectin (Ball and Morell, 2003, Annu. Rev. Plant Biol. 54, 207-233; Teltow et al., 2004, J. Expt. Bot. 55(406), 2131-2145). The group of the soluble starch synthases has several isoforms which are referred to the specialist literature as SSI, SSII, SSIII, SSIV and SSV. The association of starch synthases to the individual isoforms (SSI, SSII, SSIII, SSIV, SSV) is made with the sequence homologies of the respective protein sequences of the enzymes in question (Ball and Morell, 2003, Annu. Rev. Plant Biol. 54, 207-233). Each individual isoform of the soluble starch synthases has, in accordance with current teaching, allocated to it a specific function in the synthesis of starch. While only one isoform of SSI proteins has been detected in dicotyledonous plants, two different classes of SSII proteins have been detected in some monocotyledonous plants (for example maize), which are referred to as SSIIa and SSIIb, respectively. In monocotyledonous plants, SSIIa is expressed preferentially in the endosperm, and SSIIb preferentially in the leaf tissue (Teltow et al., 2004, J. Expt. Bot. 55(406): 2131-2145). The specific function, in particular of the individual soluble starch synthases, in the synthesis of the starch is currently not fully explained (Ball and Morell, 2003, Annu. Rev. Plant Biol. 54: 207-233).

The functional, nutritional or structure-parting characteristics of starch are also affected by the phosphate content, a noncarbon component. Here, one has to distinguish between phosphate which is bonded covalently to starch glucose molecules in the form of monoesters (referred to as starch phosphate in the context of the present invention) and phosphate in the form of starch-associated phospholipids.

The starch phosphate content varies with the plant cultivar. Thus, for example, certain maize mutants synthesize a starch with an increased starch phosphate content (waxy maize at 0.002% and high-amylose maize at 0.013%), while traditional maize varieties only contain traces of starch phosphate. Likewise, small amounts of starch phosphate are found in wheat (0.001%), while no starch phosphate was detected in oats and *Sorghum*. In waxy rice mutants, less starch phosphate (0.003%) was found than in traditional rice varieties

(0.013%). Significant amounts of starch phosphate were detected in plants which synthesize tuber or root storage starch, such as, for example, tapioca (0.008%), sweet potato (0.011%), arrow root (0.021%) or potato (0.089%). The above-cited percentages for the starch phosphate content refer in each case to the dry weight of the starch and have been determined by Jane et al. (1996, *Cereal Foods World* 41 (11): 827-832).

Starch phosphate may be present in the form of monoesters at the C2, C3 or C6 position of the polymerized glucose monomers (Takeda and Hizukuri, 1971, *Starch/Stärke* 23: 267-272). The phosphate distribution of the phosphate in starch synthesized by plants is generally distinguished by the fact that approximately 30% to 40% of the phosphate residues are bonded covalently in the C3 position and approximately 60% to 70% of the phosphate residues in the C6 position of the glucose molecules (Blennow et al., 2000, *Int. J. of Biological Macromolecules* 27: 211-218). Blennow et al. (2000, *Carbohydrate Polymers* 41: 163-174) determined a starch phosphate content which is bonded in the C6 position of the glucose molecules for a variety of starches such as, for example, potato starch (between 7.8 and 33.5 nmol per mg starch, depending on variety), starch from various *Curcuma* species (between 1.8 and 63 nmol per mg starch), tapioca starch (2.5 nmol per mg starch), rice starch (1.0 nmol per mg starch), mungbean starch (3.5 nmol per mg starch) and sorghum starch (0.9 nmol per mg starch). These authors did not detect any starch phosphate bonded in the C6 position in barley starch and starch from various waxy mutants of maize. No relationship between the genotype of a plant and the starch phosphate content has been established as yet (Jane et al., 1996, *Cereal Foods World* 41 (11): 827-832).

To date there have been described two proteins which mediate the introduction of covalent bonds of phosphate residues to starch's glucose molecules. The first protein has the enzymatic activity of an alpha-glucan, water dikinase (GWD, E.C.: 2.7.9.4) (Ritte et al., 2002, *PNAS* 99: 7166-7171), is frequently referred to as R1 in particular in the earlier scientific literature and is bound to the starch granules of storage starch in potato tubers (Lorberth et al., 1998, *Nature Biotechnology* 16: 473-477). The second protein described in the literature which catalyzes the introduction of starch phosphate into starch has the enzymatic activity of a phosphoglucan, water dikinase (PWD, E.C.: 2.7.9.5) (Kötting et al., 2005, *Plant Physiol.* 137: 2424-252, Baunsgaard et al., 2005, *Plant Journal* 41: 595-605).

One essential difference between GWD and PWD is that GWD is capable of utilizing unphosphorylated starch as its substrate, i.e. a de novo phosphorylation of unphosphorylated starch can be catalyzed by GWD, while PWD requires already phosphorylated starch as its substrate, i.e. introduces additional phosphate into already-phosphorylated starch (Kötting et al., 2005, *Plant Physiol.* 137: 2424-252, Baunsgaard et al., 2005, *Plant Journal* 41: 595-605). A further essential difference between GWD and PWD is that GWD introduces phosphate groups exclusively in the C6 position of the starch's glucose molecules, while PWD exclusively phosphorylates the C3 position of starch's glucose molecules (Ritte et al., 2006, *FEBS Letters* 580: 4872-4876).

In the reaction which is catalyzed by GWD, or PWD, the starting materials alpha-1,4-glucan (in the case of GWD) and phosphorylated alpha-1,4-glucan (in the case of PWD), respectively, adenosin triphosphate (ATP) and water are converted into the products glucan phosphate (starch phosphate), inorganic phosphate and adenosin monophosphate (Kötting et al., 2005, *Plant Physiol.* 137: 2424-252; Ritte et al., 2002, *PNAS* 99: 7166-7171).

Wheat plants which have an elevated activity of GWD proteins as the result of the expression of a GWD-encoding gene from potato are described in WO 02/34923. In comparison with corresponding wild-type plants in which no starch phosphate could be detected, these plants synthesize starch with significant amounts of starch phosphate in the C6 position of the glucose molecules.

WO 05/002359 describes the expression, in maize plants, of a potato GWD which has been optimized for the codon usage in maize plants. By means of ³¹P NMR, a total phosphate content of 0.0736% phosphate based on the amount of glucose (bonded at the C6, C3 and C2 position of the glucose molecules) of the maize starch in question was determined. If a molecular weight 98 is assumed for phosphate (H₃PO₄), a total phosphate content of approximately 7.5 nmol of phosphate per mg of starch results for the total phosphate content of 0.0736%—which has been determined in WO 05/002359—for starch isolated from transgenic maize plants. Plants which as the result of the expression of a PWD-encoding gene from *Arabidopsis thaliana* show an increased activity of a PWD protein are described in WO 05/095617. In comparison to corresponding untransformed wild type plants, these plants have an increased starch phosphate content.

An important functional characteristic, for example when processing starches in the food industry, is the swelling power. Various structural characteristics of starches, such as the amylose-/amylopectin ratio, the side chain length, the molecular weight distribution of the starch polymers, the number of branches and the amount of starch phosphate have an effect on functional characteristics, in particular on the swelling power of the starches in question (Narayana and Moorthy, 2002, *Starch/Stärke* 54: 559-592).

Amylose has long been regarded as a linear polymer consisting of α -1,4-glycosidically linked α -D-glucose monomers. However, more recent studies have demonstrated the presence of α -1,6-glycosidic branch points (approx. 0.1%) (Hizukuri and Takagi, 1984, *Carbohydr. Res.* 134: 1-10; Takeda et al., 1984, *Carbohydr. Res.* 132: 83-92).

Amylopectin constitutes a complex mixture of glucose chains with different branching patterns. In contrast to amylose, amylopectin comprises more branches. Side chains are linked via α -1,6-glycosidic linkages to the main chain of α -D-Glucose monomers, which are α -1,4-glycosidically linked. According to the literature (Voet and Voet, 1990, *Biochemistry*, John Wiley & Sons), the α -1,6-branches occur on average every 24 to 30 glucose residues. This corresponds to a degree of branching of approx. 3%-4%. The data on the degree of branching vary and depend on the origin of the starch in question (for example plant species, plant variety and the like). In typical plants used for the industrial production of starch, such as, for example, maize, wheat or potato, amylose starch accounts for approximately 20%-30% and amylopectin starch for approximately 70%-80% of the starch synthesized.

Another important difference between amylose and amylopectin is their molecular weight. While amylose, depending on the origin of the starch, has a molecular weight of 5×10^5 - 10^6 Da, the molecular weight of amylopectin is between 10^7 and 10^8 Da. The two macromolecules can be distinguished on the basis of their molecular weight and their different physical-chemical characteristics, and the simplest way of visualizing this is through their different iodine-binding characteristics.

A large number of technical applications only require amylopectin since amylopectin has a thickening action. Amylose has a gelling action and is therefore rather undesired for a

number of uses. Pure amylopectin starch makes possible a very uniform surface structure combined with high viscosity, stability and transparency. Possible applications for these starches are in papermaking, in the adhesives industry, the textiles industry, the building industry and the cosmetics industry. Furthermore, amylopectin starch is the preferred starting material for the preparation of maltodextrins as the result of their increased solubility in water, stability to dissolution and transparency in comparison with maltodextrins which are prepared from amylose-comprising starches.

In the food industry, amylopectin starches are frequently employed as stabilizers, binders and for improving texture. Amylopectin starches are particularly advantageous in the case of those processing steps at which large temperature variations occur during processing and finishing (for example freeze-thaw-stability). The use of amylopectin starches in the food industry is growing, in particular taking into consideration the increasing demand for (semi-)finished products.

GBSSI ("granule-bound starch synthase I") is involved in amylose formation. To date, plants have been described in which the activity of the granule-bound starch synthase GBSSI is reduced (Shure et al., 1983, *Cell* 35: 225-233; Hovenkamp-Hermelink et al., 1987, *Theoretical and Applied Genetics* 75: 217-221; Visser et al., 1991, *Mol. Gen. Genet.* 225: 289-296; Hergersberg, 1988, Thesis, University of Cologne; WO 92/11376). Furthermore, there are known mutants which lack a functional GBSSI gene and which therefore synthesize an amylose-free (=amylopectin) starch (Kossmann and Lloyd 2000, *Critical Reviews in Plant Sciences*, 19(3): 171-226). The endosperm of corresponding GBSSI mutant of maize is waxy in appearance, which is why the term "waxy" endosperm has been introduced as a synonym for amylose-free starches.

When describing the swelling power of starch, one must distinguish between swelling power in cold water (for example room temperature) and swelling power in warm or hot water. The swelling power of native starches in cold water is negligible, if not nonexistent, while physically modified (pregellatized, dried) starches are capable of swelling even in cold water. Preparation methods for cold water swelling starches are described for example in U.S. Pat. No. 4,280,851. In the context of the present invention, the term "swelling power" refers to the behavior of starch in warm/hot aqueous suspensions. The swelling power is routinely determined by warming starch granules in the presence of an excess of water, removing unbound water after centrifugation of the suspension and forming the quotient from the weight of the residue obtained and that of the amount of starch weighed in. When carrying out this procedure, warming the starch suspension causes crystalline regions of the starch granules to dissolve and the water molecules to intercalate into the starch granules without dissolving the structure of the starch granule itself, i.e. only a swelling of the individual starch granules takes place.

In comparison with starches from cereals, starches isolated from tubers or tuber-like tissues have a considerably higher hot-water swelling power.

For potato starches isolated from various varieties, a maximum swelling power of 74.15 g/g (variety Kufri Jyoti) at 85° C. has been determined (Singh et al., 2002, *Journal of the Science of Food and Agriculture* 82: 1376-1383), using the method of Leach et al. (1959, *Cereal Chemistry* 36: 534-544). Takizawa et al. (2004, *Brazilian Archives of Biology and Technology* 47(6): 921-931) determined a swelling power of 100 g/g for potato starch (90° C., using the method of Leach et al., above). Wheat starch isolated from various cultivars has a swelling power of 16.6 g/g to 26.0 g/g (temperature: boiling

aqueous 0.1% AgNO₃ suspension) (Yamamori and Quynh, 2000, *Theor Appl Genet.* 100: 23-38). Starch isolated from various cultivars of hull-less barley has a swelling power of 16.5 g/g or 19.3 g/g, and waxy, or amylose-free starch of various cultivars of said barley has a swelling power of 36.0 g/g to 55.7 g/g (temperature: 70° C., aqueous 0.1% AgNO₃, Yasui et al., 2002, *Starch/Stärke* 54: 179-184). For maize starch, a swelling power of 22.3 g/g has been determined, and for high-amylose maize starches a swelling power of 9.6 g/g (Hylon V), 6.1 g/g (Hylon VII) or 3.9 g/g (LAPS=Low AmyloPectin Starch) (90° C., Shi et al., 1998, *J. Cereal Sci.* 27: 289-299). U.S. Pat. No. 6,299,907 states a swelling power of 35.4 g/g for waxy maize starch. For starch isolated from various rice cultivars, a swelling power of 26.0 g/g to 33.2 g/g has been determined (Sodhi and Singh, 2003, *Food Chemistry* 80: 99-108), using the method of Leach et al. (above). Chen et al. (2003, *Starch/Stärke* 55: 203-212) determined a swelling power of approximately 25 g/g to approximately 49 g/g (95° C., aqueous suspension) for various mixtures of waxy rice starches with high-amylose rice starches. Yasui et al. (2002, *Starch/Stärke* 54: 179-184) determined a swelling power of 55.7 g/g (measured in boiling water in 0.1% aqueous silver nitrate solution) for an amylose-free rice starch.

By producing derivatives of native starches, it is possible to modify functional characteristics of the starches. Cross-linked wheat starches have a swelling power of from 6.8 g/g to 8.9 g/g, depending on the degree of crosslinking, acetylated wheat starches have a swelling power of a maximum of 10.3 g/g, and simultaneously crosslinked and acetylated wheat starches have a swelling power of 9.4 g/g, while the corresponding non-derivatized starches have a swelling power of 8.8 g/g (measured at 90° C.; Van Hung und Morita, 2005, *Starch/Stärke* 57: 413-420).

For acetylated waxy rice starches, a swelling power of approximately 30 g/g has been determined and for crosslinked waxy rice starch a swelling power of approximately 15 g/g, while corresponding non-derivatized waxy rice starch had a swelling power of approximately 41 g/g. Acetylated rice starch had a swelling power of approximately 20 g/g and crosslinked rice starch a swelling power of approximately 13 g/g, while corresponding non-derivatized rice starch had a swelling power of approximately 14 g/g (measured at 90° C., Liu et al., 1999, *Starch/Stärke* 52: 249-252). U.S. Pat. No. 6,299,907 describes crosslinked starches, where the crosslinking reaction had been carried out after preswelling the starches in question in a sodium hydroxide/sulfate solution. Depending on the degree of crosslinking, wheat starch was found to have a swelling power of from 6.8 g/g to 7.3 g/g (corresponding non-derivatized wheat starch 14.7 g/g), wheat hydroxypropyl starch a swelling power of 9.7 g/g (corresponding non-derivatized wheat starch 22.9 g/g), crosslinked maize starch a swelling power of 5.9 g/g (corresponding non-derivatized maize starch 16.7 g/g), crosslinked waxy maize starch a swelling power of 8.3 g/g (corresponding non-derivatized waxy maize starch 35.4 g/g), and crosslinked potato starch a swelling power of 6.7 g/g (corresponding non-derivatized potato starch was not specified in detail) (measurements at 95° C.). This reveals that the swelling power of starch cannot be increased substantially, if at all, by current derivatization methods.

The object of the present invention is to provide modified waxy starches with altered functional characteristics, and novel plant cells and plants which synthesize a waxy starch with altered functional characteristics, as well as methods and means for generating said plants and/or plant cells.

In particular, the altered functional characteristics consist in the fact that the modified starches have an increased hot-water swelling power.

Thus, the present invention relates to genetically modified monocotyledonous plant cells or genetically modified monocotyledonous plants whose starch has an apparent amylose content of less than 5% by weight, and which additionally have an increased activity of a protein with the enzymatic activity of a starch synthase II and additionally an increased activity of a protein with the enzymatic activity of a glucan, water dikinase in comparison with corresponding genetically not modified wild-type plant cells, or corresponding genetically not modified wild-type plants.

In this context, the genetic modification may be any genetic modification which leads to the synthesis of a starch with less than 5% by weight amylose and simultaneously to an increase in the activity of at least one protein with the activity of a starch synthase II and (simultaneously) of at least one protein with the activity of a glucan, water dikinase in genetically modified plant cells or genetically modified plants in comparison with corresponding not genetically modified wild-type plant cells or wild-type plants.

In the context of the present invention, the term “wild-type plant cell” means plant cells which act as starting material for the generation of the plant cells according to the invention, i.e. whose genetic information, with the exception of the introduced genetic modification, corresponds to that of a plant cell according to the invention.

In the context of the present invention, the term “wild-type plant” means plants which acted as starting material for the generation of the plants according to the invention, i.e. whose genetic information, with the exception of the introduced genetic modification, corresponds to that of a plant according to the invention.

In the context of the present invention, the term “corresponding” means that, when comparing several objects, the objects in question which are compared with one another are maintained under identical conditions. In the context of the present invention, the term “corresponding” in the context of wild-type plant cell or wild-type plant means that the plant cells or plants which are compared with one another were grown under identical culture conditions and have an identical (culture) age.

The term “monocotyledonous plants” refers to the monocots. Botanically, they belong to one of the three classes of the angiosperms (Magnoliophyta). In contrast to dicots, monocotyledonous plants are distinguished by the fact that the embryo typically has only one cotyledon primordium (Greek: monos=“single” and kotyledon=“cotyledon”). Moreover, they have sheathed vascular bundles, i.e. phloem and xylem are not separated by a meristem, which is why no secondary thickening of the stem is possible. This class of plants includes, inter alia, the grasses with the orders Cyperales and Poales, and a large number of other families.

In the context of the present invention, the term “increased activity of at least one protein with the (enzymatic) activity of a starch synthase II” means an increase in the expression of endogenous genes which code for proteins with the activity of a starch synthase II and/or an increase in the amount of proteins with the activity of a starch synthase II in the cells and/or an increase in the activity of proteins with the activity of a starch synthase II in the cells.

In the context of the present invention, the term “increased activity of a protein with the (enzymatic) activity of a glucan, water dikinase” means an increase in the expression of endogenous genes which code for proteins with the activity of a glucan, water dikinase and/or an increase in the amount of

proteins with the activity of a glucan, water dikinase in the cells and/or an increase in the activity of proteins with the activity of a glucan, water dikinase in the cells.

The increase in expression can be determined, for example, by measuring the amount of transcripts which code for proteins with the activity of a starch synthase II or proteins with the activity of a glucan, water dikinase. This can be done for example by northern blot analysis or by Q-PCR (quantitative transcription polymerase chain reaction).

An increase in the amount of a protein with the activity of a glucan, water dikinase means, in this context, preferably an increase in the amount of the protein in question by at least 50%, in particular by at least 70%, preferably by at least 85% and especially preferably by at least 100% in comparison to corresponding, not genetically modified cells.

An increase in the amount of protein with the activity of a glucan, water dikinase also means that plants or plant cells which contain no detectable amount of proteins with the activity of a glucan, water dikinase will, following genetic modification according to the invention, contain a detectable amount of protein with the activity of a glucan, water dikinase.

Methods for raising antibodies which specifically react to a certain protein, i.e. which specifically bind to said protein, are known to the skilled worker (see, for example, Lottspeich and Zorbas (Eds.), 1998, Bioanalytik, Spektrum akad, Verlag, Heidelberg, Berlin, ISBN 3-8274-0041-4). The raising of such antibodies can be commissioned from some companies (for example Eurogentec, Belgian). Antibodies by means of which an increase in the amount of protein with the activity of a glucan, water dikinase can be determined by means of immunological methods are described by Lorberth et al. (1998, Nature Biotechnology 16: 473-477) and Riffe et al. (2000, Plant journal 21: 387-391). Antibodies by means of which an increase in the amount of protein with the activity of a starch synthase II can be determined by means of immunological methods are described by Walter (“Untersuchungen der Expression und Funktion der Stärkesynthase II (SSII) aus Weizen (*Triticum aestivum*) [Studies into the expression and function of starch synthase II (SSII) from wheat (*Triticum aestivum*)]”, PhD Thesis at the Faculty of Biology, University of Hamburg, ISBN 3-8265-8212-8).

The amount of the activity of a protein with the activity of a glucan, water dikinase can be detected for example as described in the literature (Mikkelsen et al., 2004, Biochemical Journal 377: 525-532; Ritte et al., 2002, PNAS 99: 7166-7171).

The amount of the activity of a protein with the activity of a starch synthase II can be determined for example as described in the literature (Nishi et al., 2001, Plant Physiology 127: 459-472). A preferred method for determining the amount of the activity of a protein with the activity of a starch synthase II is described under “General Methods”.

Preferably, plant cells according to the invention or plants according to the invention have an activity of a protein with the activity of a starch synthase II which is increased by at least a factor of 2, preferably by at least a factor of 6, in comparison with corresponding genetically not modified wild-type plant cells, or wild-type plants.

The construction of proteins with the activity of a starch synthase II (ADP-glucose:1,4-alpha-D-glucan 4-alpha-D-glucosyltransferase; EC 2.4.1.21) shows a sequence of certain domains. At the N terminus, they have a signal peptide for the transport into plastids. From the N terminus toward the C terminus, there follow an N-terminal region and a catalytic domain (Li et al., 2003, Funct Integr Genomics 3, 76-85). Further analyses based on amino acid sequence alignments

(<http://hits.isb-sib.ch/cgi-bin/PFSCAN>) of various proteins with the activity of a starch synthase II revealed that these proteins have three specific domains. In the amino acid sequence shown as SEQ ID NO 4, the amino acids 322 to 351 represent domain 1, the amino acids 423 to 462 domain 2 and the amino acids 641 to 705 the domain 3. Domain 1 is encoded by the nucleotides 1190 to 1279, domain 2 by the nucleotides 1493 to 1612 and domain 3 by the nucleotides 2147 to 2350 of the nucleic acid sequence shown as SEQ ID NO 3.

In the context of the present invention, the term "protein with the activity of a starch synthase II" is understood as meaning a protein which catalyzes a glucosylation reaction in which glucose residues of the substrate ADP-glucose are transferred to alpha-1,4-linked glucan chains, with formation of an alpha-1,4-linkage ($\text{ADP-Glucose} + \{(1,4)\text{-alpha-D-glucosyl}\}(N) \rightleftharpoons \text{ADP} + \{(1,4)\text{-alpha-D-glucosyl}\}(N+1)$), where the amino acid sequence of the protein with the activity of a protein of a starch synthase II has at least 86%, preferably at least 93%, particularly preferably at least 95%, especially preferably at least 98% identity with the amino acids 322 to 351 (domain 1) of the amino acid sequence shown as SEQ ID NO 4, and at least 83%, preferably at least 86%, particularly preferably at least 95%, especially preferably at least 98% identity with the amino acids 423 to 462 (domain 2) of the amino acid sequence shown as SEQ ID NO 4 and at least 70%, preferably at least 82%, preferably 86%, particularly preferably 95%, especially preferably at least 98% identity with the amino acids 641 to 705 (domain 3) of the amino acid sequence shown as SEQ ID NO 4. Nucleic acid sequences and the corresponding amino acid sequences which have said identity with domains 1, 2 and 3 and which code for a protein with the activity of a starch synthase II are known to the skilled worker and published for example as Accession No AY133249 (*Hordeum vulgare*), Accession No AY133248 (*Aegilops tauschii*), Accession Nos XP467757, AAK64284 (*Oryza sativa*), Accession No AAK81729 (*Oryza sativa*) Accession Nos AAD13341, AAS77569, No AAD13342 (*Zea mays*), Accession No AAF13168 (*Manihot esculenta*), Accession No BAD18846 (*Phaseolus vulgaris*), Accession No CAA61241 (*Solanum tuberosum*), Accession No CAA61269 (*Pisum sativum*), Accession No AAC19119 (*Ipomea batatas*), Accession No AAF 26156 (*Arabidopsis thaliana*), Accession No AAP41030 (*Colocasia esculenta*), Accession No AAS88880 (*Ostraeococcus tauri*) or Accession No AAC17970 (*Chlamydomonas reinhardtii*). The abovementioned nucleic acid sequences and amino acid sequences coding for a protein with the activity of a starch synthase II are accessible via NCBI (<http://www.ncbi.nlm.nih.gov/entrez/>) and are expressly incorporated into the description of the present application by reference.

For the purposes of the present invention, the term "protein with the activity of a glucan, water dikinase" is understood as meaning a protein which transfers a beta-phosphate residue from ATP to starch. Starches isolated from leaves of an *Arabidopsis thaliana* sex1-3 mutant contain no detectable amounts of covalently bonded phosphate residues, but are phosphorylated in vitro by a protein with the activity of a glucan, water dikinase. This means that unphosphorylated starch, for example isolated from leaves of an *Arabidopsis thaliana* sex1-3 mutant is used as the substrate in a phosphorylation reaction which is catalyzed by a protein with the activity of a glucan, water dikinase.

A protein with the activity of a glucan, water dikinase transfers the beta-phosphate residue of ATP to starch in the C6 position of glucose, and the gamma-phosphate residue of ATP to water. Another reaction product which is generated is AMP

(adenosin monophosphate). A protein with the activity of a glucan, water dikinase is therefore also referred to as [alpha-1,4-glucan], water dikinase, or else starch: water dikinase (E.C.: 2.7.9.4; Ritte et al., 2002, PNAS 99: 7166-7171).

The phosphorylation of starch which is catalyzed by a protein with the activity of a glucan, water dikinase gives rise to additional phosphate monoester bonds exclusively in the C6 position of the glucose molecules (Ritte et al., 2006, FEBS Letters 580: 4872-4876). The catalysis of the phosphorylation reaction of a starch by a protein with the activity of a glucan, water dikinase gives rise to an intermediate phosphorylated protein in which the beta-phosphate residue of ATP is bonded covalently to an amino acid of the protein with the activity of a glucan, water dikinase (Ritte et al., 2002, PNAS 99, 7166-7171). The intermediate is formed by autophosphorylation of the protein with the activity of a glucan, water dikinase, i.e. the protein with the activity of a glucan, water dikinase itself catalyzes the reaction which leads to the intermediate. Amino acid sequences which code for proteins with the activity of a glucan, water dikinase contain a phosphohistidine domain. Phosphohistidine domains are described for example by Tien-Shin Yu et al. (2001, Plant Cell 13, 1907-1918). In the autophosphorylation of a protein with the activity of a glucan, water dikinase, a histidine residue in the phosphohistidine domain of the amino acid sequence, coding for a protein with the activity of a glucan water dikinase, is phosphorylated (Mikkelsen et al., 2004, Biochemical Journal 377: 525-532). In the protein sequence, shown for example as SEQ ID NO 2, of a protein with the activity of a glucan, water dikinase from *Solanum tuberosum*, the amino acids 1064 to 1075 constitute the phosphohistidine domain. If another amino acid is substituted for the conserved histidine residue (amino acid 1069 in the protein sequence shown for example as SEQ ID NO 2) of the phosphohistidine domain, autophosphorylation, and thus phosphorylation, of glucans by the mutagenized protein no longer takes place (Mikkelsen et al., 2004, Biochemical Journal 377: 525-532). Furthermore, a protein with the activity of a glucan, water dikinase is distinguished by the fact that it has a C-terminal nucleotide binding domain which is comprised by the amino acids 1121 to 1464 in the amino acids sequence shown for example as SEQ ID NO 2. A deletion of the nucleotide binding domain leads to inactivation of a protein with the activity of a glucan, water dikinase (Mikkelsen und Blennow, 2005, Biochemical Journal 385, 355-361). Proteins with the activity of a glucan, water dikinase have on their N terminals a carbohydrate binding domain (CBM) which is comprised by the amino acids 78 to 362 in the amino acid sequence shown as SEQ ID NO 2. Carbohydrate binding domains are distinguished inter alia by the fact that their amino acid sequences contain conserved tryptophan residues. If other amino acids are substituted for these conserved amino acid residues, the carbohydrate binding domains lose their ability of binding glucans. Thus, for example, a substitution of amino acids W139 or W194 in the amino acid sequence shown as SEQ ID NO 2 leads to a loss of function of this carbohydrate binding domain. If, however, the carbohydrate binding domain of a glucan, water dikinase is deleted (for example a deletion of amino acids 1 to 362, where the amino acids 1 to 77 in the amino acid sequence shown as SEQ ID NO 2 constitute a plastidal signal peptide), this does not lead to the inactivation of the phosphorylating activity of the enzyme (Mikkelsen et al., 2006, Biochemistry 45: 4674-4682).

Nucleic acid sequences and their corresponding amino acid sequences coding for a protein with the activity of a glucan, water dikinase are described from different species such as, for example, potato (WO 97/11188, GenBank Acc.:

AY027522, Y09533), wheat (WO 00/77229, U.S. Pat. No. 6,462,256, GenBank Acc.: AAN93923, GenBank Acc.: AR236165), rice (GenBank Acc.: AAR61445, GenBank Acc.: AR400814), maize (GenBank Acc.: AAR61444, GenBank Acc.: AR400813), Soybean (GenBank Acc.: AAR61446, GenBank Acc.: AR400815; citrus (GenBank Acc.: AY094062), *Arabidopsis* (GenBank Acc.: AF312027) and the green alga *Ostreococcus tauri* (GenBank Acc.: AY570720.1). The abovementioned nucleic acid sequences and amino acid sequences coding for a protein with the activity of a glucan, water dikinase are published inter alia by the NCBI (<http://www.ncbi.nlm.nih.gov/entrez/>) and are expressly incorporated into the description of the present application by reference.

In the context of the present invention, the term "GBSS I" is to be understood to mean any enzyme which belongs to the group of the granule-bound starch synthase of isoform I (EC 2.4.1.21).

In the context of the present invention, the term "GBSSI-Gen" is understood as meaning a nucleic acid molecule or polynucleotide (cDNA, DNA) which codes for a granule-bound starch synthase I (GBSS I). Seq ID No 7-12 comprise nucleic acid sequences or amino acid sequences which code in each case for a protein with the activity of a GBSS I from rice, wheat and maize.

Polynucleotides coding for GBSS I are described for a variety of monocotyledonous plant species such as, for example, for maize (Genbank Acc. Nos. AF079260, AF079261), wheat (Genbank Acc. Nos. AB019622, AB019623, AB019624), rice (Genbank Acc. Nos. AF092443, AF092444, AF031162), barley (Genbank Acc. Nos. X07931, X07932), *Sorghum bicolor* (Genbank Acc. No U23945) and durum wheat (Genbank Acc. No AB029063). The abovementioned nucleic acid sequences and amino acid sequences coding for a protein with the activity of a GBSS I are published inter alia by NCBI (<http://www.ncbi.nlm.nih.gov/entrez/>) and are expressly incorporated into the description of the present application by reference.

Mutants which lack a functional GBSS I gene synthesize an amylose-free starch (=waxy starch). Such mutants are described for a series of crops such as, for example, for maize (for example by Sprague et al, 1943, J. Am. Soc. Agron. 35:817-822; Shure et al. 1983, Cell 35: 225-233), rice (Sano 1984, Theor. Appl. Genet. 68: 467-473; Villareal and Juliano 1986, Starch/Staerke 38:118-119), barley (Rohde et al 1988, Nucleic Acids Res 16: 7185-7186), wheat (Nakamura et al 1995, Mol. Gen. Genet. 248: 253-259), potato (Hovenkamp-Hermelink et al. 1987, Theor. Appl. Genet. 75: 217-221) and millet (Okuno and Sakaguchi 1982, J. Hered 73: 467). The term "waxy mutant" is used synonymously, owing to the fact that, in maize, the endosperm has a waxy appearance. The GBSS I protein is also frequently referred to as "waxy protein" (Kossmann and Lloyd 2000 "Understanding and Influencing Starch Biochemistry", Critical Reviews in Plant Sciences, 19(3): 171-226).

Suitable plant cells or plants for the generation of the plant cells and plants according to the invention are those which show a reduction of the apparent amylose content in the starch synthesized by them to less than 5% by weight.

In one embodiment of the present invention, a genetic modification of the plant cells according to the invention or of the plants according to the invention is brought about by mutagenesis of one or more GBSS I genes. The nature of the mutation is of no consequence as long as it brings about a reduction, or complete diminishment, of the GBSSI activity,

and thus a reduction of the apparent amylose content of the starch present in the plants according to the invention to less than 5% by weight.

A mutation which leads to the reduction of the GBSSI activity and to the diminishment of the apparent amylose content of the starch to less than 5% by weight in the plant cells and plants according to the invention may occur spontaneously, and the plants in question can be selected and propagated with the aid of the methods described hereinbelow.

For the purposes of the present invention, a "waxy mutant" is understood as meaning a plant whose starch has an apparent amylose content of less than 5% by weight. Equally, "waxy starch" refers to a starch with an apparent amylose content of less than 5% by weight.

In the context of the present invention, the term "mutagenesis" is understood as meaning any type of introduced mutation such as, for example, deletions, point mutations (nucleotide substitutions), insertions, inversions, gene conversions or chromosomal translocations.

Agents which can be employed for generating chemically induced mutations, and the types of mutation obtained thereby as the result of the effect of the mutagens in question are described, for example, by Ehrenberg and Husain (1981, Mutation Research 86: 1-113) and Müller (1972, Biologisches Zentralblatt 91 (1): 31-48). The generation of rice mutants using gamma rays, ethylmethanesulfonate (EMS), N-methyl-N-nitrosourea or sodium azide (NaN_3) is described for example, by Jauhar and Siddiq (1999, Indian Journal of Genetics, 59 (1): 23-28), Rao (1977, Cytologica 42: 443-450), Gupta and Sharma (1990, Oryza 27: 217-219) and Satoh and Omura (1981, Japanese Journal of Breeding 31 (3): 316-326). The generation of wheat mutants using NaN_3 or maleic anhydrazide is described by Arora et al. (1992, Annals of Biology 8 (1): 65-69). An review of the generation of wheat mutants using various types of high-energy radiation and chemical agents is described by Scarascia-Mugnozza et al. (1993, Mutation Breeding Review 10:1-28). Svec et al. (1998, Cereal Research Communications 26 (4): 391-396) describe the use of N-ethyl-N-nitrosourea for the generation of mutants in triticale. The use of MMS (methylmethanesulfonic acid) and gamma radiation for the generation of millet mutants is described by Shashidhara et al. (1990, Journal of Maharashtra Agricultural Universities 15 (1): 20-23).

Monocotyledonous plant cells and plants which synthesize a starch with an apparent amylose content of less than 5% by weight (=waxy plants, or waxy plant cells) can also be generated by using what is known as insertion mutagenesis (review: Thorneycroft et al., 2001, Journal of Experimental Botany 52 (361): 1593-1601). "Insertion mutagenesis" is understood as meaning in particular the insertion of transposons, or what is known as transfer DNA (T-DNA) into a gene.

The transposons may take the form of transposons which occur naturally in a (wild-type) plant cell (endogenous transposons) or else those which do not occur naturally in said cell but have been introduced into the cell by means of recombinant methods, such as, for example, by transforming the cell (heterologous transposons). Modifying the expression of genes by means of transposons is known to the skilled worker. A review of the utilization of endogenous and heterologous transposons as tools in plant biotechnology can be found in Ramachandran and Sundaresan (2001, Plant Physiology and Biochemistry 39, 234-252). The possibility of identifying mutants in which specific genes have been inactivated by transposon insertion mutagenesis can be found in a review by Maes et al. (1999, Trends in Plant Science 4 (3), 90-96). The

generation of rice mutants with the aid of endogenous transposons is described by Hirochika (2001, *Current Opinion in Plant Biology* 4, 118-122). The identification of maize genes with the aid of endogenous retrotransposons is shown, for example, in Hanley et al. (2000, *The Plant Journal* 22 (4), 557-566). The possibility of generating mutants with the aid of retrotransposons and methods for identifying mutants are described by Kumar and Hirochika (2001, *Trends in Plant Science* 6 (3), 127-134). The activity of heterologous transposons in different species has been described both for dicotyledonous and for monocotyledonous plants, for example for rice (Greco et al., 2001, *Plant Physiology* 125, 1175-1177; Liu et al., 1999, *Molecular and General Genetics* 262, 413-420; Hiroyuki et al., 1999, *The Plant Journal* 19 (5), 605-613; Jeon and Gynheung, 2001, *Plant Science* 161, 211-219), barley (Koprek et al., 2000, *The Plant Journal* 24 (2), 253-263), *Arabidopsis thaliana* (Aarts et al., 1993, *Nature* 363, 715-717, Schmidt and Willmitzer, 1989, *Molecular and General Genetics* 220, 17-24; Altmann et al., 1992, *Theoretical and Applied Genetics* 84, 371-383; Tissier et al., 1999, *The Plant Cell* 11, 1841-1852), tomato (Belzile and Yoder, 1992, *The Plant Journal* 2 (2), 173-179) and potato (Frey et al., 1989, *Molecular and General Genetics* 217, 172-177; Knapp et al., 1988, *Molecular and General Genetics* 213, 285-290).

In principle, monocotyledonous “waxy” plant cells and plants can be generated, with the aid of both homologous and heterologous transposons, the use of homologous transposons also including those transposons which are already naturally present in the plant genome. In principle, T-DNA mutagenesis is likewise suitable for producing “waxy” plant cells and plants.

T-DNA insertion mutagenesis is based on the fact that certain segments (T-DNA) of Ti plasmids from *Agrobacterium* are capable of integrating into the genome of plant cells. The site of integration into the plant chromosome is not fixed but may take place at any position. If the T-DNA integrates in a segment of the chromosome which constitutes a gene function, this may lead to a modification of the gene expression and thus also to an altered activity of a protein encoded by the gene in question. In particular, the integration of a T-DNA into the coding region of a gene frequently means that the protein in question can no longer be synthesized in active form, or not at all, by the cell in question. The use of T-DNA insertions for the generation of mutants is described, for example, for *Arabidopsis thaliana* (Krysan et al., 1999, *The Plant Cell* 11, 2283-2290; Atipiroz-Leehan and Feldmann, 1997, *Trends in Genetics* 13 (4), 152-156; Parinov and Sundaresan, 2000, *Current Opinion in Biotechnology* 11, 157-161) and rice (Jeon and An, 2001, *Plant Science* 161, 211-219; Jeon et al., 2000, *The Plant Journal* 22 (6), 561-570). Methods for identifying mutants which have been generated with the aid of T-DNA insertion mutagenesis are described, inter alia, by Young et al., (2001, *Plant Physiology* 125, 513-518), Parinov et al. (1999, *The Plant Cell* 11, 2263-2270), Thomeycroft et al. (2001, *Journal of Experimental Botany* 52, 1593-1601), and McKinney et al. (1995, *The Plant Journal* 8 (4), 613-622).

Mutations in the corresponding gene can be found with the aid of methods with which the skilled worker is familiar. For example, it is possible to employ molecular analyzes based on hybridizations with probes (“Southern blot”), on the amplification by means of polymerized chain reaction (PCR), on the sequencing of suitable genomic nucleic acid fragments and the search for individual nucleotides substitutions. An example of a method of identifying mutations with the aid of hybridization patterns is the search for restriction fragment

length polymorphisms (RFLP) (Nam et al., 1989, *The Plant Cell* 1: 699-705; Leister and Dean, 1993, *The Plant Journal* 4 (4): 745-750). A PCR based method is, for example, the analysis of amplified fragment length polymorphisms (AFLP) (Castiglioni et al., 1998, *Genetics* 149: 2039-2056; Meksem et al., 2001, *Molecular Genetics and Genomics* 265: 207-214; Meyer et al. 1998, *Molecular and General Genetics* 259: 150-160). The use of amplified fragments which have been cleaved with restriction endonucleases (“cleaved amplified polymorphic sequences”, CAPS) is a further possibility of identifying mutations (Konieczny and Ausubel, 1993, *The Plant Journal* 4: 403-410; Jarvis et al., 1994, *Plant Mol. Biol.* 24: 685-687; Bachem et al., 1996, *The Plant Journal* 9 (5): 745-753). Methods of determining SNPs have been described by, inter alia, Qi et al. (2001, *Nucleic Acids Research* 29 (22): 116), Drenkard et al. (2000, *Plant Physiology* 124: 1483-1492) and Cho et al. (1999, *Nature Genetics* 23: 203-207). Particularly suitable methods are those which permit a large number of plants to be studied for mutations in certain genes within a short period of time. Such a method, known as TILLING (“targeting induced local lesions in genomes”) has been described by McCallum et al. (2000, *Plant Physiology* 123: 439-442).

The skilled worker knows that the above-described mutations are, as a rule, recessive mutations. To manifest the waxy phenotype, it is therefore necessary to generate true-breeding (homozygous) plant cells or plants. Methods of generating true-breeding plants are known to the skilled worker.

Homozygous “waxy” mutants can be identified by staining the starch with iodine. To this end, starch-comprising tissue samples (for example endosperm, pollen) are stained with iodine solution and studied for example under the microscope. Waxy starches stain brown (in comparison with the blue staining of the wild type).

In a further embodiment of the present invention, the introduction of one or more foreign nucleic acid molecules/polynucleotides, their presence and/or the expression of one or more foreign nucleic acid molecules/polynucleotides lead to the inhibition of the expression of endogenous genes which code for the GBSS I protein and to a reduction of the apparent amylose content of the starch present in the plant cell according to the invention, or plant according to the invention, to less than 5% by weight.

This can be done by various methods with which the skilled worker is familiar. These methods include, for example, the expression of a suitable antisense RNA, or of a double-stranded RNA, the provision of molecules or vectors which confer a cosuppression effect, the expression of a suitably constructed ribozyme which specifically cleaves transcripts which code for GBSSI, or what is known as “in-vivo mutagenesis”. Furthermore, the reduction of the GBSSI activity/activities and/or the reduction of the gene expression of the GBSSI gene in the plant cells can also be brought about by the simultaneous expression of sense and antisense RNA molecules of the specific target gene to be repressed, preferably the GBSSI gene. These methods are known to the skilled worker.

In addition, it is known that the formation of double-stranded RNA of promoter sequences in trans can bring about methylation and transcriptional inactivation of homologous copies of this promoter in planta (Mette et al., 2000, *EMBO J.* 19: 5194-5201).

To inhibit the gene expression by means of antisense or cosuppression technology, for example, it is possible to employ a DNA molecule which comprises all of the GBSSI coding sequence including any flanking sequences present, or else DNA molecules which only comprise parts of the coding

sequence, where these parts must be long enough to bring about an antisense effect, or cosuppression effect, in the cells. Generally suitable are sequences with a minimum length of 15 bp, preferably with a minimum length of 20-30 bp, especially preferably with a length of 100-500 bp, and, for highly efficient antisense or cosuppression inhibition, in particular sequences with a length of more than 500 bp.

Also suitable for antisense or cosuppression approaches is the use of polynucleotide sequences with a high degree of identity with the endogenous sequences which are present in the plant cell and which encode GBSSI. The minimum identity should be greater than approximately 65%. The use of sequences with identities of at least 90%, in particular between 95% and 100%, is to be preferred.

To achieve an antisense effect, or a cosuppression effect, it is furthermore also feasible to use introns, i.e. from noncoding regions of genes which code for GBSSI.

The use of intron sequences for inhibiting the expression of genes which code for starch biosynthesis proteins has been described in WO 97/04112, WO 97/04113, WO 98/37213, WO 98/37214.

The skilled worker knows how to achieve an antisense effect and a cosuppression effect. The method of cosuppression inhibition has been described, for example, by Jorgensen (1990, Trends Biotechnol. 8: 340-344), Niebel et al. (1995, Top. Microbiol. Immunol. 197: 91-103), Flavell et al. (1995, Curr. Top. Microbiol. Immunol. 197: 43-46), Palauqui and Vaucheret (1995, Plant Mol. Biol. 29: 149-159), Vaucheret et al. (1995, Mol. Gen. Genet. 248: 311-317), de Borne et al. (1994, Mol. Gen. Genet. 243: 613-621).

Furthermore, a reduction of the GBSSI activity in the plant cells can also be brought about by the simultaneous expression of sense and antisense RNA molecule of the specific target gene to be repressed, preferably the GBSSI gene.

This can be achieved for example by using chimeric constructs which comprise "inverted repeats" of the target gene in question, or parts of the target gene. The chimeric constructs code for sense and antisense RNA molecules of the target gene in question. Sense and antisense RNA are synthesized simultaneously in planta as one RNA molecule, it being possible for sense and antisense RNA to be separated from each other by a spacer, to form a double-stranded RNA molecule (RNAi technology).

It has been demonstrated that the introduction of inverted-repeat DNA constructs into the genome of plants is a highly effective method for repressing the genes corresponding to the inverted-repeat DNA constructs (Waterhouse et al., 1998, Proc. Natl. Acad. Sci. USA 95, 13959-13964; Wang and Waterhouse, 2000, Plant Mol. Biol. 43, 67-82; Singh et al., 2000, Biochemical Society Transactions 28 (6), 925-927; Liu et al., 2000, Biochemical Society Transactions 28 (6), 927-929; Smith et al., 2000, Nature 407, 319-320; WO 99/53050). Sense and antisense sequences of the target gene, or target genes, may also be expressed separately from one another by means of identical or different promoters (Nap et al, 6th International Congress of Plant Molecular Biology, 18-24 Jun. 2000, Quebec, Poster S7-27, Lecture Session S7).

The expression of ribozymes for reducing the activity of specific enzymes in cells is also known to the skilled worker and described, for example, in EP-B1 0321201. The expression of ribozymes in plant cells has been described for example by Feyter et al. (1996, Mol. Gen. Genet. 250: 329-338).

Moreover, the reduction of the GBSSI activity and/or the reduction of the apparent amylose content of the starch present in the plant cells to less than 5% by weight may also be achieved by what is known as "in-vivo" mutagenesis,

where an RNA-DNA oligonucleotide hybrid ("chimeroplast") is introduced into cells by means of transforming cells (Kipp et al., Poster Session at the 5th International Congress of Plant Molecular Biology, 21-27 Sep. 1997, Singapore; R. A. Dixon and C. J. Arntzen, Meeting report regarding Metabolic Engineering in Transgenic Plants, Keystone Symposia, Copper Mountain, Colo., USA, 1997, TIBTECH 15: 441-447; WO 95/15972; Kren et al., 1997, Hepatology 25: 1462-1468; Cole-Strauss et al., 1996, Science 273: 1386-1389; Beetham et al., 1999, PNAS 96: 8774-8778).

Part of the DNA component of the RNA-DNA oligonucleotide is homologous with a polynucleotide sequence of an endogenous GBSSI gene, but comprises a mutation in comparison with the polynucleotide acid sequence of an endogenous GBSSI gene or comprises a heterologous region which is surrounded by the homologous regions. Owing to base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous polynucleotide, followed by homologous recombination, the mutation or heterologous region present in the DNA component of the RNA-DNA oligonucleotide can be transferred into the genome of a plant cell.

Thus, the reduction of the GBSSI activity in the plant cells can also be achieved by generating double-stranded RNA molecules of GBSSI genes. To this end, it is preferred to introduce, into the genome of plants, inverted repeats of DNA molecules which are derived from nucleotide sequences formed by GBSSI genes or cDNAs formed by such genes, where the DNA molecules to be transcribed are under the control of a promoter which governs the expression of said RNA molecules.

A further possibility of reducing the activity of proteins in plant cells or plants is the method of what is known as immunomodulation. It is known that an expression in planta of antibodies which specifically recognize a plant protein results in a reduction of the activity of said proteins in corresponding plant cells or plants as the result of the formation of a protein/antibody complex (Conrad and Manteufel, 2001, Trends in Plant Science 6: 399-402; De Jaeger et al., 2000, Plant Molecular Biology 43: 419-428; Jobling et al., 2003, Nature Biotechnology 21: 77-80).

All the abovementioned methods are based on the introduction of one or more foreign nucleic acid molecules into the genome of plant cells or plants and are therefore suitable in principle for the generation of plant cells according to the invention and plants according to the invention.

The reduction of the expression can be determined for example by measuring the amount of transcripts which code for the enzymes in question, for example by means of Northern blot analysis or quantitative RT-PCR.

The reduction of the amount of GBSSI protein can be determined for example by immunological methods such as Western blot analysis, ELISA ("enzyme linked immunosorbent assay") or RIA ("radio immune assay").

A reduction in the GBSSI activity in the plant cells, or plants, according to the invention can also be detected indirectly via quantifying of the reaction product of the GBSSI protein, amylose. The skilled worker knows a multiplicity of methods for determining the amylose content in plant starches. For cereals, in particular rice, the apparent amylose content is preferably determined by a method similar to that of Juliano (1971, Cereal Science Today 16 (10): 334-340), as described further below in the chapter "Materials and Methods".

In a further embodiment for generating the plant cells according to the invention or the plants according to the invention, it is possible to use, instead of a wild-type plant cell

or wild-type plant, a mutant which is distinguished by the fact that it already synthesizes a starch with an apparent amylose content of less than 5% by weight and/or which has an increased activity of a protein with the activity of a glucan, water dikinase and/or an increased activity of a protein with the activity of a starch synthase II. These mutants may be either spontaneously occurring mutants or else those which have been generated by the targeted use of mutagens. Possibilities of generating such mutants have been described hereinabove.

The present invention furthermore comprises a genetically modified monocotyledonous plant cell, or plant, according to the invention whose genetic modification consists in the introduction of at least one foreign nucleic acid molecule into the genome of the plant used for the transformation.

As the result of the introduction of a foreign nucleic acid molecule, the genetic information of the plant cells according to the invention or plants according to the invention are altered. The presence of at least one foreign nucleic acid molecule leads to an altered "phenotype". Here, "altered phenotype" means a measurable alteration of one or more cellular functions. For example, the genetically modified plant cells according to the invention and the genetically modified plants according to the invention show, as the result of the presence or, in the case of expression of introduced foreign nucleic acid molecules, an increase in the activity of a protein with the activity of a glucan, water dikinase and an increase in the activity of a protein with the activity of a starch synthase II and/or a reduction of the activity of a protein with the activity of a GBSSI.

In the context of the present invention, the term "foreign nucleic acid molecule" is understood as meaning a molecule which either does not occur naturally in the plant cells used for the transformation, or which does not occur naturally in the specific spatial arrangement in the plant cells used for the transformation, or which is located at a locus in the genome of the plant cell used for the transformation at which it does not occur naturally. The foreign nucleic acid molecule is preferably a recombinant molecule which consists of various elements whose combination or specific spatial arrangement does not occur naturally in plant cells. Thus, recombinant nucleic acid molecules may, for example, besides nucleic acid molecules which code for a protein with the activity of a glucan, water dikinase and/or a protein with the activity of a starch synthase II and/or a nucleic acid which brings about a reduction in the activity of a GBSSI, have additional nucleic acid sequences which are not naturally present in combination with the abovementioned nucleic acid molecules. The abovementioned additional nucleic acid sequences which are present on a recombinant nucleic acid molecule in combination with a nucleic acid molecule coding for protein with the activity of a glucan, water dikinase and/or protein with the activity of a starch synthase II and/or with a nucleic acid which is suitable for mediating a reduction in the activity of a protein with the activity of a GBSSI may be any sequences. They may be for example genomic and/or plant nucleic acid sequences. Preferably, these additional nucleic acid sequences are regulatory sequences (promoters, termination signals, enhancers), particularly preferably regulatory sequences which are active in plant tissue; especially preferably tissue-specific regulatory sequences.

Methods of generating recombinant nucleic acid molecules are known to the skilled worker and comprise genetic engineering methods such as, for example, the linking of nucleic acid molecules by ligation, genetic recombination or the de-novo synthesis of nucleic acid molecules (see, for example, Sambrook et al., *Molecular Cloning, A Laboratory*

Manual, 3rd edition (2001) Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y., ISBN: 0879695773; Ausubel et al., *Short Protocols in Molecular Biology*, John Wiley & Sons; 5th edition (2002), ISBN: 0471250929).

In the context of the present invention, the term "genome" is understood as meaning the totality of the hereditary material present in a plant cell. The skilled worker knows that not only the nucleus, but other compartments too (for example plastids, mitochondria) comprise hereditary material.

In principle, a foreign nucleic acid molecule can be any nucleic acid molecule which brings about, in the plant cell or plant, an increase in the activity of a protein with the activity of a glucan, water dikinase and of a protein with the activity of a starch synthase II and a reduction in the activity of a protein with the activity of a GBSSI.

In a preferred embodiment, the foreign nucleic acid molecules coding for a protein with the activity of a glucan, water dikinase take the form of the already-mentioned nucleic acid molecules from the various plant species, which nucleic acid molecules are known to the skilled worker. Particularly preferred in this context are nucleic acid molecules coding for a protein with the activity of a glucan, water dikinase from potato, especially preferred is a protein with the activity of a glucan, water dikinase which has the amino acid sequence shown in SEQ ID NO 2 or is encoded by the nucleic acid sequence shown in SEQ ID NO 1.

In a further preferred embodiment, the foreign nucleic acid molecules coding for a protein with the activity of a starch synthase II take the form of the already-mentioned nucleic acid molecules from the various plant species, which nucleic acid molecules are known to the skilled worker. Particularly preferred in this context are nucleic acid molecules coding for a protein with the activity of a starch synthase II from wheat, especially preferred is a protein with the activity of a starch synthase II which has the amino acid sequence shown in SEQ ID NO 4 or is encoded by the nucleic acid sequence shown in SEQ ID NO 3.

A further preferred embodiment takes the form of nucleic acid molecules coding for a protein with the activity of a starch synthase II from rice, especially preferably a protein with the activity of a starch synthase II which has the amino acid sequence shown in SEQ ID NO 6 or is encoded by the nucleic acid sequence shown in SEQ ID NO 5.

In a further preferred embodiment, the foreign nucleic acid molecules coding for a protein with the activity of a GBSSI take the form of the already-mentioned nucleic acid molecules from the various plant species, which nucleic acid molecules are known to the skilled worker. Particularly preferred in this context are nucleic acid molecules coding for a protein with the activity of a GBSSI from rice, especially preferred is a protein with the activity of a GBSSI which has the amino acid sequence shown in SEQ ID NO 8 or is encoded by the nucleic acid sequence shown in SEQ ID NO 7.

A further preferred embodiment takes the form of nucleic acid molecules coding for a protein with the activity of a GBSSI from wheat, especially preferably a protein with the activity of a GBSSI which has the amino acid sequence shown in SEQ ID NO 10 or is encoded by the nucleic acid sequence shown in SEQ ID NO 9.

A further preferred embodiment takes the form of nucleic acid molecules coding for a protein with the activity of the GBSSI from maize, especially preferably a protein with the activity of a GBSSI which has the amino acid sequence shown in SEQ ID NO 12 or is encoded by the nucleic acid sequence shown in SEQ ID NO 11.

In a further embodiment, the plant cells and plants according to the invention are homozygous for the waxy mutation(s) and thus synthesize a starch whose apparent amylose content is less than 5% by weight.

In the context of the present invention, the term “homozygous for the waxy mutation(s)” is understood as meaning that the plant breeds true for the non-functional GBSSI genes. To the skilled worker, homozygosity means that, within the hereditary material of a cell, all alleles regarding a particular trait are identical, that is to say two or more identical copies of a certain gene are present on the two chromatids of a chromosome, which chromatids comprise the gene. They are homozygous (=breed true) for this gene and, when selfed, pass on the trait in question to all progeny. The skilled worker knows that polyploid plants such as, for example, wheat may, under certain circumstances, require three non-functional GBSSI alleles (on the subgenomes A, B and D) in homozygous form in order to manifest the waxy phenotype.

The foreign nucleic acid molecules introduced, for the purposes of genetic modification, into the plant cells or plant which manifest the waxy phenotype may take the form of a single nucleic acid molecule or more nucleic acid molecules. They may take the form of nucleic acid molecules which comprise nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and nucleic acid sequences which code for a protein with the activity of a starch synthase II, but also nucleic acid molecules in which the nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II are present on different nucleic acid molecules. For example, the nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II may be present simultaneously in a vector, plasmid or in linear nucleic acid molecules (“dual construct”) or else be components of two vectors, plasmids or linear nucleic acid molecules which are separate in each case.

If the nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II are present in two separate nucleic acid molecules, they can be introduced into the genome of the plant cell or plant either simultaneously (“cotransformation”) or else one after the other, i.e. with a chronological interval (“supertransformation”). The separate nucleic acid molecules may also be introduced into different individual plant cells or plants of a species. Thereby it is possible to generate plant cells or plants in which the activity of either at least one protein with the activity of a glucan, water dikinase or else at least one protein with the activity of a starch synthase II is elevated. Plants according to the invention can then be generated by subsequently hybridizing those plants in which the activity of a protein with the activity of a glucan, water dikinase is elevated with those in which the activity of a protein with the activity of a starch synthase II is elevated. The parameters for the selection of plants which are used for the process steps in question are defined further below.

In a further embodiment, the waxy phenotype of the plant cells or plants according to the invention is brought about by introducing one or more recombinant nucleic acid molecules suitable for reducing the GBSSI activity.

The foreign nucleic acid molecules introduced, for the purposes of genetic modification, into the wild-type plant cell or plant may take the form of a single nucleic acid molecule or more nucleic acid molecules. They may therefore take the form of nucleic acid molecules which comprise nucleic acid

sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II and additionally to nucleic acid sequences which are suitable for inhibiting the activity of the GBSSI activity (triple construct). Equally, they may also take the form of nucleic acid molecules in which the nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II are present on different nucleic acid molecules, where one or the other of these two nucleic acid molecules additionally comprises nucleic acid sequences which are suitable for inhibiting the activity of the GBSSI activity. Alternatively, they may also take the form of nucleic acid molecules in which the nucleic acid sequences which code for a protein with the activity of a glucan, water dikinase and the nucleic acid sequences which code for a protein with the activity of a starch synthase II are present on one nucleic acid molecule and the nucleic acid molecules which are suitable for inhibiting the GBSSI activity are present on a different nucleic acid molecule (3 variants of one dual construct and one simple construct).

In a further embodiment, they may also take the form of three different nucleic acid molecules, where one comprises nucleic acid sequences which code for a glucan, water dikinase protein, another one comprises nucleic acid sequences coding for a starch synthase II and a further one comprises nucleic acid sequences which are suitable for inhibiting the GBSSI activity (3 simple constructs).

The nucleic acid molecules which are suitable for generating the plant cells or plants according to the invention may be present for example in a vector, plasmid or in linear nucleic acid molecules.

If the constructs to be used for the generation of plant cells or plants according to the invention are present in two or three separate nucleic acid molecules, they can be introduced into the genome of the plant cell or plant either simultaneously (“cotransformation”) or else one after the other, i.e. with a chronological interval (“supertransformation”). The separate nucleic acid molecules may also be introduced into different individual plant cells or plants of a species. Thereby it is possible to generate plant cells or plants in which the activity of either at least one protein with the activity of a glucan, water dikinase and/or at least one protein with the activity of a starch synthase II is elevated and/or at least one protein with the activity of a GBSSI activity is reduced to such an extent that the starch synthesized by the plant cells or plants has an apparent amylose content of less than 5% by weight. Plants according to the invention can then be generated by subsequently hybridizing the plants.

Furthermore, it is also possible to generate plants in which the activity of at least one protein with the (enzymatic) activity of a GBSSI is reduced to such an extent that the starch synthesized by the plant cells or plants has an apparent amylose content of less than 5% by weight and which, in a further step, by crossing with plants in which the activity of at least one protein with the activity of a starch synthase II is elevated, leads to plant cells or plants according to the invention.

In the event that one or more nucleic acid molecules which comprise nucleic acid sequences suitable for increasing the activity of at least one protein with the activity of a glucan, water dikinase and/or of a starch synthase II in the plant cells and reducing the activity of a GBSSI in the plant cells to such an extent that the starch synthesized by the cells has an apparent amylose content of less than 5%, are introduced into the genome of the plant cells in one methodological step/simul-

taneously, the plants according to the invention may be selected directly among the plants to which the transformation gives rise.

In a further embodiment, the plant cells according to the invention and the plants according to the invention comprise that at least one foreign nucleic acid molecule codes for a protein with the activity of a starch synthase II and a second foreign nucleic acid molecule codes for a protein with the activity of a glucan, water dikinase. In a further embodiment, the plant cells according to the invention of the plants according to the invention comprise that a first foreign nucleic acid molecule codes for a protein with the activity of a glucan, water dikinase and a second foreign nucleic acid molecule codes for a protein with the activity of a starch synthase II.

A multiplicity of techniques is available for introducing DNA into a plant host cell. These techniques comprise the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, the fusion of protoplasts, the injection, the electroporation of DNA, the introduction of the DNA by means of the biolistic approach, and other possibilities.

The use of the agrobacteria-mediated transformation of plant cells has been studied intensively and has been described, inter alia, in EP 120516; Hoekema (In: The Binary Plant Vector System, Offsetdrukkerij Kanters B. V. Alblasterdam (1985), Chapter V); Fraley et al., Crit. Rev. Plant Sci. 4: 1-46) and by An et al. (1985, EMBO J. 4: 277-287).

The transformation of monocotyledonous plants by means of vectors based on *Agrobacterium* transformation has also been described (Chan et al. 1993, Plant Mol. Biol. 22: 491-506; Hiei et al., 1994, Plant J. 6, 271-282; Deng et al, 1990, Science in China 33: 28-34; Wilmink et al., 1992, Plant Cell Reports 11: 76-80; May et al., 1995, Bio/Technology 13: 486-492; Conner and Domisse, 1992, Int. J. Plant Sci. 153: 550-555; Ritchie et al, 1993, Transgenic Res. 2: 252-265). Alternative methods for the transformation of monocotyledonous plants are the transformation by means of the biolistic approach (Wan and Lemaux, 1994, Plant Physiol. 104: 37-48; Vasil et al., 1993, Bio/Technology 11: 1553-1558; Ritala et al., 1994, Plant Mol. Biol. 24: 317-325; Spencer et al., 1990, Theor. Appl. Genet. 79: 625-631), the transformation of protoplasts, the electroporation of partially permeabilized cells or the introduction of DNA by means of glass fibers. The transformation of maize, in particular, is described repeatedly in the literature (cf., for example, WO95/06128, EP0513849, EP0465875, EP0292435; Fromm et al., 1990, Biotechnology 8: 833-844; Gordon-Kamm et al., 1990, Plant Cell 2: 603-618; Koziel et al., 1993, Biotechnology 11: 194-200; Moroc et al., 1990, Theor. Appl. Genet. 80: 721-726).

This successful transformation of other cereal species has also been described, for example in the case of barley (Wan and Lemaux, s.o.; Ritala et al., s.o.; Krens et al., 1982, Nature 296: 72-74) and wheat (Nehra et al., 1994, Plant J. 5: 285-297; Becker et al., 1994, Plant Journal 5: 299-307). All the above methods are suitable within the scope of the present invention.

Plant cells and plants whose starch has an amylose content of less than 5% by weight and which are genetically modified as the result of the introduction of a gene coding for a protein with the activity of a glucan, water dikinase and/or a gene coding for a protein with the activity of a starch synthase II can be distinguished from wild-type plant cells, or wild-type plants, inter alia by the fact that they comprise at least one foreign nucleic acid molecule which does not occur naturally in wild-type plant cells, or wild-type plants, or by the fact that such a molecule is present at a location in the genome of the plant cell according to the invention or in the genome of the

plant according to the invention at which it does not occur in wild-type plant cells, or wild-type plants, i.e. in a different genomic environment. Furthermore, such plant cells according to the invention or plants according to the invention can be distinguished from wild-type plant cells, or wild-type plants, by the fact that they comprise at least one copy of the foreign nucleic acid molecule stably integrated in their genome, if appropriate additionally to copies of such a molecule which are naturally present in the wild-type plant cells, or wild-type plants. If the foreign nucleic acid molecule(s) which has been introduced into the plant cells according to the invention or plants according to the invention takes the form of additional copies, besides molecules which naturally occur in the wild-type plant cells, or wild-type plants, the plant cells according to the invention and the plants according to the invention can be distinguished from wild-type plant cells, or wild-type plants, in particular by the fact that this additional copy, or these additional copies, is/are located at locations in the genome where it does not occur, or they do not occur, in wild-type plant cells or wild-type plants. This can be verified for example with the aid of a Southern blot analysis.

The plant cells according to the invention or the plants according to the invention can furthermore be preferably distinguished from wild-type plant cells, or wild-type plants, by at least one of the following features: if a foreign nucleic acid molecule which has been introduced is heterologous with regard to the plant cell or plant, then the plant cells according to the invention, or plants according to the invention, comprise transcripts of the nucleic acid molecules which have been introduced. These transcripts can be detected for example by Northern blot analysis or by RT-PCR (reverse transcription polymerase chain reaction).

Plant cells according to the invention or plants according to the invention which express an antisense transcript and/or an RNAi transcript can be detected for example with the aid of specific nucleic acid probes which are complementary to the RNA which codes for the protein (and which occurs naturally in the plant cell). Preferably, the plant cells according to the invention and the plants according to the invention comprise a protein which is encoded by a nucleic acid molecule which has been introduced. This protein can be detected for example by immunological methods, in particular by Western blot analysis.

Preferably, the plant cells according to the invention or the plants according to the invention comprise a protein which is encoded by a nucleic acid molecule which has been introduced. This protein can be detected for example by immunological methods, in particular by Western blot analysis.

If a foreign nucleic acid molecule which has been introduced is homologous with regard to the plant cell or plant, then the plant cells according to the invention, or the plants according to the invention, can be distinguished from wild-type plant cells, or wild-type plants, for example on the basis of the additional expression of the foreign nucleic acid molecules which have been introduced. The plant cells according to the invention and the plants according to the invention preferably comprise transcripts of the foreign nucleic acid molecules. This can be detected for example by Northern blot analysis or with the aid of what is known as quantitative PCR.

A further subject matter of the present invention relates to genetically modified monocotyledonous plant cells or genetically modified monocotyledonous plants which synthesize a modified starch in comparison with starch isolated from corresponding, not genetically modified wild-type plant cells, or isolated from corresponding not genetically modified wild-type plants.

The invention furthermore relates to genetically modified monocotyledonous plants which comprise plant cells according to the invention. Such plants can be generated from plant cells according to the invention by means of regeneration.

The plants according to the invention may, in principle, take the form of any monocotyledonous plants. Preferably, they take the form of monocotyledonous crop plants, i.e. plants which are grown by man for the purposes of nutrition or for technical, in particular industrial, purposes.

In a further embodiment, the plants according to the invention take the form of starch-storing monocotyledonous plants, or the plant cells according to the invention are derived from a starch-storing plant.

In the context of the present invention, the term "starch-storing plant" means all plants with plant parts which comprise a storage starch such as, for example, maize, rice, wheat, rye, oats, barley, sago, taro and millet/sorghum.

In a preferred embodiment, the present invention relates to monocotyledonous plants of the (systematic) family Poaceae. These plants particularly preferably take the form of rice, maize or wheat plants. These plants very particularly preferably take the form of rice plants.

In the context of the present invention, the term "wheat plants" means plant species of the genus *Triticum* or plants which have originated from crosses with plants of the genus *Triticum*, particularly plant species of the genus *Triticum* which are grown in agriculture for commercial purposes, or plants which have originated from crosses with plants of genus *Triticum*, with *Triticum aestivum* being especially preferred.

In the context of the present invention, the term "maize plants" means plant species of the genus *Zea*, particularly plant species of the genus *Zea*, which are grown in agriculture for commercial purposes, particularly preferably *Zea mays*.

In the context of the present invention, the term "rice plant" means plant species of the genus *Oryza*, particularly plant species of the genus *Oryza*, which are grown in agriculture for commercial purposes, particularly preferably *Oryza sativa*.

The present invention also relates to propagation material of monocotyledonous plants comprising genetically modified plant cells.

Here, the term "propagation material" comprises those parts of the plant which are suitable for generating progeny via the vegetative or sexual route. Examples which are suitable for vegetative propagation are cuttings, callus cultures, rhizomes or tubers. Other propagation material comprises for example fruits, seeds, seedlings, protoplasts, cell cultures and the like.

In a further embodiment, the present invention relates to plant parts capable of being harvested of plants according to the invention such as fruits, storage roots, roots, flowers, buds, shoots or stems, preferably seeds or kernels, these parts which are capable of being harvested comprising plant cells according to the invention.

In a further embodiment, the genetically modified monocotyledonous plant cells according to the invention are distinguished by the fact that they synthesize a (waxy) starch with elevated hot-water swelling power and an amylose content of less than 5% by weight.

In a preferred embodiment, the genetically modified monocotyledonous plant cell is distinguished by the fact that it comprises a waxy starch with an elevated hot-water swelling power of between 60 to 100 g/g.

Particularly preferred in this context is a hot-water swelling power of between 70 and 95 g/g, very particularly preferred of between 80 and 95 g/g and extraordinarily preferred of between 80 and 90 g/g.

A further subject matter of the present invention relates to a method of generating a genetically modified monocotyledonous plant, where

a) a plant cell is genetically modified, the genetic modification comprising the following steps i to iii:

i) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells,

ii) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells,

iii) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to a reduction in the activity of a protein with the activity of a GBSSI in comparison with corresponding not genetically modified wild-type plant cells,

where steps i to iii can be carried out in any desired sequence, individually or simultaneously as any desired combination of steps i to iii,

b) a plant is regenerated from plant cells of step a);

c) if appropriate, further plants are generated with the aid of the plants of step b), where, if appropriate, plant cells are isolated from plants in accordance with steps b) or c) and the method steps a) to c) are repeated until a plant has been generated which has an increased activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells and reduced activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells and reduced activity of a protein with the activity of a GBSSI in comparison with corresponding not genetically modified wild-type plant cells.

The present invention furthermore also relates to a method of generating a genetically modified plant, in which a plant cell whose starch has an amylose content of less than 5% by weight is genetically modified, where genetic modification comprises the following steps a) and b) in any desired sequence, individually or simultaneously:

a) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells,

b) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells, and

c) a plant is regenerated from plant cells of step a) and b);

d) if appropriate, further plants are generated with the aid of the plants from steps a) and b),

where, if appropriate, plant cells are isolated from plants according to step a) or b) and the method steps a) to c) are repeated until a plant has been generated which comprises a foreign nucleic acid molecule coding for a protein with the activity of a starch synthase II and a foreign nucleic acid molecule coding for a protein with the activity of a glucan, water dikinase.

A preferred subject matter of the present invention relates to methods of generating a monocotyledonous plant, wherein

a) a plant cell is genetically modified, where the genetic modification comprises the following steps i to iii in any

desired sequence, or any desired combinations of the following steps i to iii are carried out individually or simultaneously

- i) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells,
 - ii) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells,
 - iii) introduction, into the plant cell, of a genetic modification, where the genetic modification leads to a reduction in the activity of a protein with the activity of a GBSSI in comparison with corresponding not genetically modified wild-type plant cells,
- b) a plant is regenerated from plant cells comprising the genetic modification in accordance with steps
- i) a) i
 - ii) a) ii
 - iii) a) iii
 - iv) a) i and a) ii,
 - v) a) i and a) iii,
 - vi) a) ii and a) iii, or
 - vii) a) i and a) ii and a) iii
- c) there is introduced, into plant cells from plants in accordance with step
- i) b) i, a genetic modification in accordance with step a) ii,
 - ii) b) i, a genetic modification in accordance with step a) iii,
 - iii) b) i, a genetic modification in accordance with step a) ii and simultaneously a genetic modification in accordance with step a) iii,
 - iv) b) ii, a genetic modification in accordance with step a) i,
 - v) b) ii, a genetic modification in accordance with step a) iii,
 - vi) b) ii, a genetic modification in accordance with step a) i and simultaneously a genetic modification in accordance with step a) iii,
 - vii) b) iii, a genetic modification in accordance with step a) i,
 - viii) b) iii, a genetic modification in accordance with step a) ii,
 - ix) b) iii, a genetic modification in accordance with step a) i and simultaneously a genetic modification in accordance with step a) ii,
 - x) b) iv, a genetic modification in accordance with step a) iii,
 - xi) b) v, a genetic modification in accordance with step a) ii, or
 - xii) b) vi, a genetic modification in accordance with step a) i and the plant is regenerated,
- d) there is introduced, into plant cells of plants in accordance with step
- i) c) i, a genetic modification in accordance with step a) iii,
 - ii) c) ii, a genetic modification in accordance with step a) ii,
 - iii) c) iv, a genetic modification in accordance with step a) iii,
 - iv) c) v, a genetic modification in accordance with step a) ii,
 - v) c) vii, a genetic modification in accordance with step a) ii,
 - vi) c) vii, a genetic modification in accordance with step a) i, or
 - vii) c) ix, a genetic modification in accordance with step a) ii and a plant is regenerated,

if appropriate, further plants are generated with the aid of the plants in accordance with one of steps b) vii, c) iii, c) vi, c) x, c) xi, c) xii or in accordance with any of steps d) i to d) vii.

The genetic modifications introduced in accordance with step a) into the plant cell may, in principle, take the form of any type of modification which leads to an increase in the activity of a protein with the activity of a starch synthase II and/or which leads to the increase in the activity of a protein with the activity of a glucan, water dikinase and/or which leads to the reduction in the activity of a protein with the activity of a GBSSI.

The regeneration of the plants in accordance with steps b) to e) of the methods according to the invention can be accomplished by methods known to the skilled worker (for example described in "Plant Cell Culture Protocols", 1999, ed. by R. D. Hall, Humana Press, ISBN 0-89603-549-2).

The generation of further plants of the methods according to the invention can be accomplished for example by vegetative propagation (for example via cuttings, tubers or via callus culture and regeneration of intact plants) or by generative propagation. Generative propagation preferably takes place in a controlled manner, i.e. selected plants with specific properties are crossed with each other and propagated. The selection is preferably accomplished in such a way that the further plants (which are generated, depending on the method, in accordance with step c) or step d) or step e)) have the modifications introduced in the preceding steps.

The parameters for the selection of the plant cells or plants according to the invention which can be generated by crossing or by transformation are detailed hereinbelow: in the case where exclusively at least one protein with the activity of a glucan, water dikinase is increased, suitable plants or plant cells are those which have a phosphate content in the C6 position of the starch of at least 2.5 nmol per mg starch. In the case where exclusively at least one protein with the activity of a starch synthase II is increased, suitable plants or plant cells are those which have an SSII activity which is increased by at least a factor of 2 over the SSII activity in the plant cells or plants which are used for introducing the nucleic acid molecule(s) according to the invention or used for crossing.

In the case where at least one protein with the activity of a glucan, water dikinase and at least one protein with the activity of a starch synthase II are increased, suitable plants or plant cells are those which have a phosphate content in the C6 position of the starch of at least 2.5 nmol per mg starch and additionally an SSII activity which is increased by at least a factor of 2 over the SSII activity in the plant cells or plants which are used for introducing the nucleic acid molecule(s) according to the invention or used for crossing.

in the case where the GBSSI activity is reduced, or waxy mutants are employed, suitable plants are those which have an apparent amylose content of less than 5% by weight when the mutation is present in homozygous form.

Another suitable selection criterion is the level of the starch phosphate content in the C6 position. Plants which are preferably selected are those which comprise the genetic modification in accordance with step a) and b) and whose starch phosphate content is at least 2.5 nmol C6P/mg starch and whose starch has an apparent amylose content of less than 5% by weight.

In the method according to the invention for the generation of genetically modified plants, the genetic modifications for generating the genetically modified plant cells according to the invention can be effected simultaneously or in successive steps. In this context, it is not critical whether the same method is used for successive genetic modifications which lead to an increased activity of a protein with the activity of a

starch synthase II as for the genetic modification which leads to an increased activity in a protein with the activity of a glucan, water dikinase and/or for the genetic modification which leads to a reduced activity of a protein with the activity of a GBSSI.

Various selection criteria may be chosen for selecting the plants according to the invention, or those plants which are used for further modifications.

In a further embodiment of the method according to the invention for the generation of a genetically modified plant, step c) is followed by a method step c)-1, in which plants are selected whose starch has an apparent amylose content of less than 5% by weight and an increased activity in a protein with the activity of a starch synthase II in accordance with step a)i) and/or has an increased activity of a protein with the activity of a glucan, water dikinase in accordance with step a)ii). The selected plants are then used for the further method steps.

In a further embodiment of the method according to the invention for the generation of a genetically modified plant according to the invention, at least one foreign nucleic acid molecule codes for a protein with the activity of a glucan, water dikinase from potato, wheat, rice, maize, soybean, citrus, *Curcuma* or *Arabidopsis*. Preferably, at least one foreign nucleic acid molecule codes for a protein with the activity of a glucan, water dikinase from potato and especially preferably for a protein which has the amino acid sequence shown in SEQ ID NO 2 or which is encoded by the nucleic acid sequence shown in SEQ ID NO 1. References for nucleic acid sequences coding for proteins with the activity of a glucan, water dikinase from the abovementioned plants have already been detailed further above.

In a further embodiment of the method according to the invention for generating a genetically modified plant according to the invention, at least one foreign nucleic acid molecule codes for a protein with the activity of a starch synthase II from wheat, barley, *Aegilops*, rice, maize, cassava, bean, potato, pea, sweet potato, *Arabidopsis*, taro, *Ostreococcus* or *Chlamydomonas*. Preferably, at least one foreign nucleic acid molecule codes for a protein with the activity of a starch synthase II from wheat, in particular Seq ID No 3. References for nucleic acid sequences coding for proteins with the activity of a starch synthase II from the abovementioned plants have already been detailed further above.

As already described above for foreign nucleic acid molecules introduced into a plant cell or plant for the purposes of genetic modification, the nucleic acid molecule(s) in step a) of the method according to the invention for the generation of a genetically modified plant whose starch has an amylose content of less than 5% by weight may take the form of a single nucleic acid molecule or a plurality of nucleic acid molecules. Thus, the foreign nucleic acid molecules coding for a protein with the activity of a starch synthase II, or coding for a protein with the activity of a glucan, water dikinase, may be present together on a single nucleic acid molecule or else they may be present in separate nucleic acid molecules. If the nucleic acid molecules coding for a protein with the activity of a starch synthase II and coding for a protein with the activity of a glucan, water dikinase are present in a plurality of nucleic acid molecules, these nucleic acid molecules may be introduced into a plant cell either simultaneously or in successive steps.

In a further embodiment of the method according to the invention for the generation of a genetically modified plant according to the invention, at least one foreign nucleic acid molecule codes for a protein with the activity of a GBSSI from a monocotyledonous plant, preferably from rice, wheat, barley, maize, *Aegilops*, sorghum or oats.

References for the abovementioned nucleic acid sequences coding for proteins with the activity of a GBSSI from the abovementioned plants have already been detailed further above.

5 Preferably, at least one foreign nucleic acid molecule codes for a protein with the activity of a GBSSI from rice and especially preferably for a protein which is encoded by the nucleic acid sequence shown in SEQ ID NO 7 or by the amino acid sequence shown in SEQ ID NO 8.

10 In a further preferred embodiment, at least one foreign nucleic acid molecule codes for a protein with the activity of a GBSSI from wheat and especially preferably for a protein which is encoded by the amino acid sequence shown in SEQ ID NO 9 or shown in SEQ ID NO 10.

15 In a further preferred embodiment, at least one foreign nucleic acid molecule codes for a protein with the activity of a GBSSI from maize and especially preferably for a protein which is encoded by the nucleic acid sequence shown in SEQ ID NO 11 or by the amino acid sequence shown in SEQ ID NO 12.

20 Here, the foreign nucleic acid molecule brings about the inhibition of the activity of a GBSS I and thus the synthesis of a starch with an amylose content of less than 5% by weight. What has been said above regarding the use of the nucleic acids in question for the generation of plant cells or plants according to the invention also applies here analogously.

25 The foreign nucleic acid molecule(s) used for the genetic modification may take the form of one combined or of a plurality of separate nucleic acid constructs, in particular of what are known as simple, dual or triple constructs. Thus, the foreign nucleic acid molecule may be what is known as a "triple construct", which is understood as meaning a single vector for the transformation of plants which comprises not only the genetic information for inhibiting the expression of an endogenous GBSSI gene, but also the information for the overexpression of one or more SSII genes and for the overexpression of one or more GWD genes.

30 A basic principle in the construction of the foreign nucleic acid molecules for inhibiting the GBSSI activity is the use of antisense, cosuppression, ribozyme and double-stranded RNA constructs and of sense constructs, which use leads to a reduction in the expression of endogenous genes which code for GBSSI and which leads to a simultaneous increase in the activity of the proteins with the activities of an SSII and/or of a GWD.

35 In this context, the foreign nucleic acid molecules may be introduced into the genome of the plant cell either simultaneously ("cotransformation") or else one after the other, i.e. in chronological succession ("supertransformation").

40 The foreign nucleic acid molecules may also be introduced into different individual plants of one species. In this way, it is possible to generate plants in which the activity of a protein with the activity of a GBSSI is reduced and/or the activity of a protein with the activity of an SSII or GWD is increased. Subsequently, crosses may then be made to generate plants in which the activity of a protein with the activity of a GBSSI is reduced and the activity of a protein with the activity of an SSII and a GWD is increased.

45 In the context of the present invention, the term "identity" is understood as meaning the number of amino acids/nucleotides which agree (identity) with other proteins/nucleic acids, expressed in percent.

50 Preferably, the identity regarding a protein with the activity of a starch synthase II is determined by comparing the amino acid sequences detailed under SEQ ID NO 4 or SEQ ID NO 6, or the identity regarding a nucleic acid molecule coding for a protein with the activity of a starch synthase II by comparing

the nucleic acid sequences detailed under SEQ ID NO 3 or SEQ ID NO 5, and the identity regarding a protein with the activity of a glucan, water dikinase by comparing the amino acid sequence detailed in SEQ ID NO 2, or the identity regarding a nucleic acid molecule coding for a protein with the activity of a glucan, water dikinase by comparing the nucleic acid sequence detailed in SEQ ID NO 1, and the identity regarding a nucleic acid molecule coding for a protein with the activity of a GBSSI by comparing the nucleic acid sequences detailed in SEQ ID NO 7 or SEQ ID NO 9 or SEQ ID NO 11, or the amino acid sequences detailed in SEQ ID NO 8 or SEQ ID NO 10 or SEQ ID NO 12, with other proteins/nucleic acids with the aid of computer programs.

If sequences which are compared with each other are different in length, the identity is to be determined in such a way that the number of amino acids/nucleotides which the shorter sequence shares with the longer sequence determines the percentage identity. The identity is preferably determined by means of known computer programmes which are publicly available such as, for example, ClustalW (Thompson et al., *Nucleic Acids Research* 22 (1994), 4673-4680). ClustalW is made publicly available by Julie Thompson (Thompson@EMBL-Heidelberg.DE) and Toby Gibson (Gibson@EMBL-Heidelberg.DE), European Molecular Biology Laboratory, Meyerhofstrasse 1, D 69117 Heidelberg, Germany. ClustalW can likewise be downloaded from various internet pages, inter alia the IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P.163, 67404 Illkirch Cedex, France; ftp://ftp-igbmc.u-strasbg.fr/pub/) and the EBI (ftp://ftp.ebi.ac.uk/pub/software/) and all mirrored EBI internet pages (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK).

To determine the identity between proteins described within the scope of the present invention and other proteins, it is preferred to employ the ClustalW computer program version 1.8. The following parameters are to be set: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3, GAPOpen=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET, ENDGAPS(OFF), NOPGAP, NOHGAP.

To determine the identity between for example the nucleotide sequence of the nucleic acid molecules described within the scope of the present invention and the nucleotide sequence of other nucleic acid molecules, it is preferred to employ the ClustalW computer program version 1.8. The following parameters are to be set: KTUPLE=2, TOPDIAGS=4, PAIRGAP=5, DNAMATRIX:IUB, GAPOpen=10, GAPEXT=5, MAXDIV=40, TRANSITIONS: unweighted.

Identity furthermore means that functional and/or structural equivalence exists between the nucleic acid molecules in question or the proteins encoded by them. The nucleic acid molecules which are homologous to the above-described molecules and which are derivatives of these molecules will, as a rule, take the form of variations to these molecules which are modifications with the same biological function. They may take the form of naturally occurring variations, for example sequences from other species or else of mutations, where it is possible that these mutations have occurred naturally or else have been introduced by specific mutagenesis. Furthermore, the variations may take the form of synthetically generated sequences. The allelic variants may take the form of naturally occurring variants or else of synthetically generated variants or variants which have been generated by recombinant DNA technology. A specific form of derivatives are for example nucleic acid molecules which deviate from

the nucleic acid molecules described within the scope of the present invention as the result of the degeneracy of the genetic code.

Within the scope of the present invention, the term "hybridization" means hybridization under traditional hybridization conditions, preferably under stringent conditions as are described for example in Sambrook et al., (*Molecular Cloning, A Laboratory Manual*, 3rd edition (2001) Cold Spring Harbour Laboratory Press, Cold Spring Harbour, N.Y.; ISBN: 0879695773). Particularly preferably, "to hybridize" means hybridization under the following conditions:

Hybridization Buffer:

2×SSC; 10×Denhardt solution (Ficoll 400+PEG+BSA; ratio 1:1:1); 0.1% SDS; 5 mM

EDTA; 50 mM Na₂HPO₄; 250 µg/ml herring sperm DNA; 50 µg/ml tRNA; or

25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS

Hybridization Temperature:

T=65 to 68° C.

Wash buffer: 0.1×SSC; 0.1% SDS

Wash temperature: T=65 to 68° C.

Nucleic acid molecules which hybridize with the above-mentioned molecules can be isolated for example from genomic libraries or from cDNA libraries. The identification and isolation of such nucleic acid molecules may be accomplished using the abovementioned nucleic acid molecules or parts of these molecules, or using the reverse complements of these molecules, for example by means of hybridization by standard methods, or by amplification by means of PCR.

Hybridization probes which can be used for isolating a nucleic acid sequence coding for a protein with the activity of a starch synthase II or with the activity of a glucan, water dikinase or with the activity of a GBSSI are, for example, nucleic acid molecules with exactly the nucleotide sequences, or essentially the nucleotide sequences, detailed in SEQ ID NO 3 or SEQ ID NO 5 (starch synthase II) or in SEQ ID NO 1 (glucan, water dikinase) or in SEQ ID NO 7, 9 or 11 (GBSSI), or parts of these sequences.

The fragments used as hybridization probe may also take the form of synthetic fragments or oligonucleotides which have been generated with the aid of the customary synthetic techniques and whose sequence agrees essentially with that of a nucleic acid molecule described within the scope of the present invention. When genes which hybridize with the nucleic acid sequences described within the scope of the present invention have been identified and isolated, a determination of the sequence and an analysis of the characteristics of the proteins encoded by this sequence should be carried out to verify that they are proteins with the activity of a starch synthase II or the activity of a glucan, water dikinase or the activity of a GBSSI, respectively.

The molecules which hybridize with the nucleic acid molecules described within the scope of the present invention comprise in particular fragments, derivatives and allelic variants of the abovementioned nucleic acid molecules. In the context of the present invention, the term "derivative" means that the sequences of these molecules differ from the sequences of the above-described nucleic acid molecules at one or more positions and that they have a high degree of identity with these sequences. The deviations from the above-described nucleic acid molecules may have been generated for example by deletion, addition, substitution, insertion or recombination.

To express nucleic acid molecules according to the invention which code for a protein with the activity of starch synthase II and/or a protein with the activity of a glucan, water dikinase and/or a protein with the activity of a GBSSI, these

molecules are preferably linked with regulatory DNA sequences which ensure transcription in plant cells. These include in particular promoters. In general, any promoter which is active in plant cells is suitable for expression.

The promoter may be selected in such a way that expression takes place constitutively or else only in a certain tissue, at a certain point in time of plant development or at a point in time determined by external factors. The promoter may be homologous or heterologous both with regard to the plant and with regard to the nucleic acid molecule.

Examples of suitable promoters are the 35S RNA promoter of the Cauliflower Mosaic Virus and the maize ubiquitin promoter, the rice ubiquitin promoter (Liu et al., *Plant Science* 165, (2003), the rice actin promoter (Zhang, et al., *Plant Cell* 3:1150-1160, 1991), the Cassava Vein Mosaic Virus (CVMV) promoter (Verdaguer et. al., *Plant Mol. Biol.* 31: 1129-1139), the maize histone H₃C4 promoter (U.S. Pat. No. 6,750,378) or the Cestrum YLCV promoter (Yellow Leaf Curling Virus; WO 01 73087; Stavolone et al., 2003, *Plant Mol. Biol.* 53, 703-713) for the purposes of constitutive expression. A promoter which ensures expression only in photosynthetically active tissues may also be used, for example the ST-LS1 promoter (Stockhaus et al., *Proc. Natl. Acad. Sci. USA* 1987, 84: 7943-7947; Stockhaus et al., *EMBO J.* 1989, 8: 2445-2451), or for endosperm-specific expression, the wheat HMW promoter, the *Vicia faba* USP promoter (Fiedler et al., 1993, *Plant Mol. Biol.* 22: 669-679; Baumlein et al., 1991, *Mol. Gen. Genet.* 225: 459-467), the bean phaseolin promoter, promoters of zein genes from maize (Pedersen et al., 1982, *Cell* 29: 1015-1026; Quatroccio et al., 1990, *Plant Mol. Biol.* 15: 81-93), a glutelin promoter (Leisy et al., 1990, *Plant Mol. Biol.* 14: 41-50; Zheng et al., 1993, *Plant J.* 4: 357-366; Yoshihara et al., 1996, *FEBS Lett.* 383: 213-218), a globulin promoter (Nakase et al., 1996, *Gene* 170(2): 223-226), a prolamin promoter (Qu and Takaiwa, 2004, *Plant Biotechnology Journal* 2(2): 113-125). However, it is also possible to use promoters which are activated only at a point in time which is determined by external factors (see, for example, WO 93/07279). Promoters which are also of interest may be promoters of heat-shock proteins, which can make simple induction possible. Furthermore, it is possible to use seed-specific promoters, such as, for example, the *Vicia faba* USP promoter (see above).

A termination sequence (polyadenylation signal) may also be present; this serves to add a poly-A tail to the transcript. The poly-A tail is assumed to have a function in the stabilization of the transcripts. Such elements are described in the literature (cf. Gielen et al., 1989, *EMBO J.* 8: 23-29) and may be exchanged as desired.

It is also possible for intron sequences to be present between the promoter and the coding region. Such intron sequences may lead to the stability of the expression and to an increased expression in plants (Callis et al., 1987, *Genes Devel.* 1: 1183-1200; Luehrsen and Walbot 1991, *Mol. Gen. Genet.* 225: 81-93; Rethmeier et al. 1997, *Plant Journal.* 12(4): 895-899; Rose and Beliakoff 2000, *Plant Physiol.* 122 (2): 535-542; Vasil et al., 1989, *Plant Physiol.* 91: 1575-1579; Xu et al. 2003, *Science in China Series C Vol.* 46(6): 561-569). Examples of suitable intron sequences are the first intron of the maize sh1 gene, the first intron of the maize poly-ubiquitin gene 1, the first intron of the rice EPSPS gene, or one of the first two introns of the *Arabidopsis* PAT1 gene.

A further embodiment of the present invention relates to a method of generating a genetically modified monocotyledonous plant according to the invention, wherein a plant cell whose starch has an apparent amylose content of less than 5% by weight

a) is genetically modified, where the genetic modification leads to an increase in the activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells;

5 b) a plant is regenerated from plant cells of step a);

c) if appropriate, further plants are generated with the aid of the plants in accordance with step b), and

10 d) plants obtained in accordance with step b) or c) are crossed with a plant which shows an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells.

A further embodiment of the present invention relates to a method of generating a genetically modified monocotyledonous plant according to the invention, wherein a plant cell whose starch has an apparent amylose content of less than 5% by weight

15 a) is genetically modified, where the genetic modification leads to an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding not genetically modified wild-type plant cells;

b) a plant is regenerated from plant cells of step a);

c) if appropriate, further plants are generated with the aid of the plants in accordance with step b), and

20 d) plants obtained in accordance with step b) or c) are crossed with a plant which shows an increase in the enzymatic activity of a protein with the activity of a starch synthase II in comparison with corresponding not genetically modified wild-type plant cells.

30 A further embodiment of the present invention relates to a method of generating a genetically modified monocotyledonous plant according to the invention, wherein a plant cell is genetic modified, where

a) i) the genetic modification leads to an increase in the activity of a protein with the activity of a glucan, water dikinase;

a) ii) a further genetical modification is carried out which leads to an increase in the activity of a protein with the activity of a starch synthase II

40 in comparison with corresponding not genetically modified wild-type plant cells; where steps a) i) and ii) can be carried out in any desired sequence,

b) a plant is regenerated from plant cells of step a) i) and ii);

45 c) if appropriate, further plants are generated with the aid of the plants in accordance with step b), and

d) plants obtained in accordance with steps a) to c) are crossed with a plant whose starch thus has an amylose content of less than 5% by weight in comparison with corresponding not genetically modified wild-type plant cells.

50 In the three last-mentioned methods of generating a genetically modified plant, the plants may be genetically modified in accordance with step a), as already described above. The regeneration of plants in accordance with step b) and the generation of further plants in accordance with steps c) and d) have also been detailed further above.

A plant which is crossed in accordance with step d) of the first two embodiments with plants or progeny of the plants obtained from step b) or c) may be any plant which shows an increase in the activity of a protein with the activity of a starch synthase II or an increase in the activity of a protein with the activity of a glucan, water dikinase in comparison with corresponding wild-type plants. The increase in the activity of a protein with the activity of a starch synthase II, or a protein with the activity of a glucan, water dikinase, may have been brought about by any modification which leads to an increase in the activity of the proteins in question in the corresponding plants. These plants may take the form of mutants or of plants

which have been modified by recombinant methods. The mutants may take the form of spontaneously (naturally) occurring mutants or else of those which have been generated by the targeted use of mutagens (such as, for example, chemical agents, ionizing radiation) or recombinant methods (for example transposon activation tagging, T-DNA activation tagging, in vivo mutagenesis).

Plants which are preferably used for crosses in the two last-mentioned methods according to the invention are those with an activity of a protein with the activity of a starch synthase II which is increased by at least 3-fold, preferably 6-fold, preferably at least 8-fold and particularly preferably at least 10-fold in comparison with corresponding genetically not modified wild-type plants.

Such plants in question with an increased activity of a protein with the activity of a glucan, water dikinase are used for crosses in the two last-mentioned methods according to the invention are preferably plants which synthesize a starch with a starch phosphate content of at least 2.5 nmol C6P/mg starch.

In a preferred embodiment, methods according to the invention are used for generating a genetically modified plant for generating plants according to the invention or for generating plants which have the characteristics of plants according to the invention.

The present invention also relates to plants obtainable by methods according to the invention.

Surprisingly, it has been found that plant cells according to the invention and plants according to the invention whose starch has an apparent amylose content of less than 5% by weight and an increase in the activity of a protein with the activity of a starch synthase II and an increase in the activity of a protein with the activity of a glucan, water dikinase synthesize a modified starch. The fact that starch synthesized by plant cells according to the invention or plants according to the invention has an increased hot-water swelling power was particularly surprisingly. The increased hot-water swelling power of starches which can be isolated from plant cells according to the invention and plants according to the invention imparts to the starches according to the invention properties which make them better suited to certain applications than traditional starches. If starch is employed for example as a thickener, the increased hot-water swelling power of the starch means that considerably less starch is required for achieving the same thickening power.

A further subject matter of the present invention relates to modified starch with an apparent amylose content of less than 5% by weight and an increased hot-water swelling power. The hot-water swelling power of modified starch according to the invention is increased preferably by at least the factor 1.5, particularly preferably by at least the factor 2, especially preferably by at least the factor 2.5 and very particularly preferably by at least the factor 3 in comparison with starch isolated from corresponding not genetically modified wild-type plant cells or isolated from corresponding not genetically modified wild-type plants.

Methods for determining the hot-water swelling power are known to the skilled worker and described in the literature (for example Leach et al., 1959, Cereal Chemistry 36: 534-544). A method to be used by preference in connection with the present invention for determining the hot-water swelling power is described further below in "General Methods".

A further subject matter of the present invention relates to modified starch, isolated from a monocotyledonous plant cell or from a monocotyledonous plant, with an apparent amylose content of 5% by weight and which has a hot-water swelling power of from at least 60 g/g, preferably of from 60 to 100 g/g,

particularly preferably of from 70 to 95 g/g, especially preferably of from 80 to 95 g/g and specifically preferably of from 80 to 90 g/g.

A further subject matter of the present invention relates to modified starch, isolated from rice plant cells or rice plants, with an apparent amylose content of 5% by weight and a hot-water swelling power of from at least 60 g/g, preferably of from 60 to 100 g/g, particularly preferably of from 70 to 95 g/g, especially preferably of from 80 to 95 g/g and specifically preferably of from 80 to 90 g/g.

Starch synthesized by genetically modified plant cells according to the invention or genetically modified plants according to the invention preferably has an increased content of phosphate in the C6 position of the starch. Here, the starch phosphate content of starch isolated from plant cells according to the invention and plants according to the invention is markedly higher than the starch phosphate content which would be expected after making crosses on the basis of the total of the starch phosphate contents of the parent plants in question.

The amount of the starch phosphate bound in the C6 position of the glucose molecules can be determined by methods known to the skilled worker, such as, for example, photometrically by means of coupled enzyme assays or by means of ³¹P NMR, following the method described by Kasemusuan and Jane (1996, Cereal Chemistry 73: 702-707). In the context of the present invention, the amount of starch phosphate bound in the C6 position of the glucose molecules is preferably determined as described in "General Methods".

A further preferred subject matter of the present invention relates to modified starch according to the invention which has been isolated from a monocotyledonous plant cell or from a monocotyledonous plant and which has a starch phosphate content bound in the C6 position of the glucose molecules of the starch of at least 1.5 nmol per mg starch, particularly preferably of at least 2.5 nmol per mg starch. This modified starch according to the invention particularly preferably takes the form of maize, rice or wheat starch.

In a further embodiment of the present invention, the modified starches according to the invention take the form of native starches.

In the context of the present invention, the term "native starch" means that the starch is isolated by methods known to the skilled worker from plants according to the invention, harvestable plant parts according to the invention, starch-storing parts according to the invention or plant propagation material according to the invention.

The present invention also relates to modified starch according to the invention obtainable from plant cells according to the invention or plants according to the invention, from propagation material according to the invention or from harvestable plant parts according to the invention, or obtainable from plants which have been generated using a method according to the invention for generating a genetically modified plant.

Plant cells or plants which synthesize a modified starch according to the invention are likewise subject matter of the present invention.

The present invention furthermore relates to a method of generating a modified starch comprising the step of extracting the starch from a plant cell according to the invention or a plant according to the invention, from propagation material according to the invention of such a plant and/or from harvestable plant parts according to the invention of such a plant, preferably from starch-storing parts according to the invention of such a plant. Preferably, such a method also comprises the step of harvesting the plants or plant parts which have

been grown and/or the propagation material of these plants before extracting the starch, and particularly preferably furthermore the step of growing plants according to the invention before harvesting.

Methods for extracting the starch from plants, or from starch-storing parts of plants, are known to the skilled worker. Furthermore, methods for extracting the starch from various starch-storing plants have been described, for example in *Starch: Chemistry and Technology* (Ed.: Whistler, BeMiller and Paschall (1994), 2nd edition, Academic Press Inc. London Ltd; ISBN 0-12-746270-8; see, for example, chapter XII, page 412-468: Mais and sorghum starches: production; by Watson; chapter XIII, page 469-479: Tapioca, Arrowroot and Sago starches: production; by Corbishley and Miller; chapter XIV, page 479-490: potato starch: production and uses; by Mitch; chapter XV, page 491 to 506: wheat starch: production, modification and uses; by Knight and Oson; and chapter XVI, page 507 to 528: rice starch: production and uses; by Rohmer and Klem; maize starch: Eckhoff et al., 1996, *Cereal Chem.* 73: 54-57, the extraction of maize starch on the industrial scale is generally accomplished by what is known as wet milling). Devices which are usually employed in processes for extracting starch from plant material are separators, decanters, hydrocyclones, spray dryers and fluidized-bed dryers.

In the context of the present invention, the term "starch-storing parts" are understood as meaning those parts of a plant in which starch, in contrast to transitory leaf starch, is stored as a reserve for surviving for longer periods. Preferred starch-storing plant parts are, for example, tubers, storage roots and grains, particularly preferred are grains comprising an endosperm, especially preferred are grains comprising an endosperm from maize, rice or wheat plants.

In a preferred embodiment, methods according to the invention for preparing a modified starch are used for preparing a starch according to the invention.

Modified starch obtainable by a process according to the invention for preparing modified starch is also a subject matter of the present invention.

The use of plant cells according to the invention or plants according to the invention for preparing a modified starch is also subject matter of the present invention.

The skilled worker knows that the properties of starch can be altered for example via thermal, chemical, enzymatic or mechanical derivatization. Derivatized starches are particularly suitable for a variety of uses in the food and/or nonfood sector. The starches according to the invention are better suited as starting material for the preparation of derivatized starches than conventional starches since they comprise a higher proportion of reactive functional groups, for example as a result of the higher starch phosphate content. As the result of the increased hot-water swelling power of starches according to the invention, the derivatization processes can furthermore be carried out at higher temperatures without the starch granule structure being damaged to a substantial degree.

The present invention therefore also relates to processes for preparing a derivatized starch, wherein modified starch according to the invention is subsequently derivatized. The present invention furthermore relates to a derivatized starch prepared by one of the known processes.

In the context of the present invention, the term "derivatized starch" is understood as meaning a modified starch according to the invention whose properties have been altered with the aid of chemical, enzymatic, thermal or mechanical processes after the starch has been isolated from plant cells.

In another embodiment of the present invention, the derivatized starch according to the invention is heat- and/or acid-treated starch.

In a further embodiment, the derivatized starches take the form of starch ethers, in particular starch alkyl ethers, O-allyl ethers, hydroxyl alkyl ethers, O-carboxymethyl ethers, nitrogen-containing starch ethers, phosphate-containing starch ethers or sulfur-containing starch ethers.

In a further embodiment, the derivatized starches take the form of crosslinked starches.

In a further embodiment, the derivatized starches take the form of starch graft polymers.

In a further embodiment, the derivatized starches take the form of oxidized starches.

In a further embodiment, the derivatized starches take the form of starch esters, in particular starch esters which have been introduced into the starch using organic acids. They particularly preferably take the form of what are known as phosphate starches, nitrate starches, sulfate starches, xanthate starches, acetate starches or citrate starches.

The derivatized starches according to the invention are suitable for a variety of uses in the pharmaceutical industry, in the food sector and/or in the nonfood sector. Methods of preparing derivatized starches according to the invention are known to the skilled worker and extensively described in the general literature. A review of the preparation of derivatized starches is found for example in Orthoefer (in *Corn, Chemistry and Technology*, 1987, eds. Watson and Ramstad, Chapter 16: 479-499).

Derivatized starch obtainable by the process according to the invention for preparing a derivatized starch is likewise subject matter of the present invention.

The use of modified starches according to the invention for the preparation of derivatized starch is furthermore subject matter of the present invention.

The present invention also comprises products comprising a starch according to the invention.

The present invention also comprises mixtures comprising the starch according to the invention.

Starch-storing parts of plants are frequently processed into flours. Examples of parts of plants from which flours are prepared are, for example, tubers of potato plants and grains of cereal plants. To prepare flours from cereal plants, the endosperm-containing grains of these plants are ground and sieved. Starch is a main constituent of the endosperm. In other plants which comprise no endosperm, but other starch-storing parts such as, for example, tubers or roots, flour is frequently prepared by comminuting, drying and subsequently grinding the storage organs in question. The starch of the endosperm or present in starch-storing parts of plants accounts for a considerable proportion of the flour which is prepared from the plant parts in question. The properties of flours are therefore also influenced by the starch present in the flour in question.

Plant cells according to the invention and plants according to the invention synthesize an altered starch in comparison with corresponding not genetically modified wild-type plant cells, or not genetically modified wild-type plants. Flours prepared from plant cells according to the invention, plants according to the invention, propagation material according to the invention or harvestable parts according to the invention therefore have altered properties. The properties of flours may also be influenced by mixing starch with flours or by mixing flours with different properties.

A further subject matter of the present invention therefore relates to flours comprising a starch according to the invention.

A further subject matter of the present invention relates to flours which can be prepared from plant cells according to the invention, plants according to the invention, starch-storing parts of plants according to the invention, from propagation material according to the invention or from harvestable plant parts according to the invention. Preferred starch-storing parts of plants according to the invention for the preparation of flours are tubers, storage roots and grains which comprise an endosperm. Particularly preferred in the context of the present invention are grains from plants of the (systematic) family Poaceae; especially preferably, grains are obtained from maize, rice or wheat plants.

In the context of the present invention, the term "flour" is understood as meaning a powder which can be obtained by grinding plant parts. If appropriate, plant parts are dried and sieved prior to grinding.

On account of the starch according to the invention present in them, flours according to the invention are distinguished by the fact that they have an increased hot-water swelling power. This is desirable for example in the processing of flours in the food industry for a multiplicity of applications, in particular in the production of baked good.

A preferred subject matter of the present invention relates to flours prepared from grains of a monocotyledonous waxy plant, which flours have a hot-water swelling power of at least 25 g/g, preferably of from 25 to 50 g/g, particularly preferably of from 30 to 45 g/g and especially preferably of from 35 to 45 g/g.

In this context, the determination of the hot-water swelling power of flours is effected analogously to the above-described method for determining the hot-water swelling power for starch, with the difference that flours are employed in place of starch. A preferred method of determining the hot-water swelling power of flours is described in "General Methods".

A further subject matter of the present invention is a process for the preparation of flours, comprising the step of grinding plant cells according to the invention, plants according to the invention, parts of plants according to the invention, starch-storing parts of plants according to the invention, propagation material according to the invention or harvestable material according to the invention.

Flours can be produced by grinding starch-storing parts of plants according to the invention. The skilled worker knows how to produce flours. Preferably, a process for the production of flours also comprises the step of harvesting the plants or plant parts which are grown and/or the propagation material and/or the starch-storing parts of these plants before grinding, and particularly preferably furthermore the step of growing plants according to the invention before harvesting.

Products comprising a flour according to the invention are likewise subject matter of the present invention.

In a further embodiment of the present invention, the process for the production of flours comprises the processing of plants according to the invention, of starch-storing parts of plants according to the invention, of propagation material according to the invention or of harvestable material according to the invention prior to grinding.

In this context, processing may be a heat treatment and/or a drying step. A heat treatment followed by the drying of the heat-treated material is employed for example in the production of flours from storage roots or tubers such as, for example, from potato tubers, before grinding takes place. The comminution of plants according to the invention, of starch-storing parts of plants according to the invention, of propagation material according to the invention or of harvestable material according to the invention before grinding may like-

wise constitute processing within the meaning of the present invention. The removal of plant tissue before grinding, such as, for example, hulling the grains, also constitutes processing before grinding within the meaning of the present invention.

In a further embodiment of the present invention, the process for the preparation of flours comprises processing the mill base after grinding. In this context, the mill base may be sieved after grinding in order to prepare various types of flours.

The present invention also comprises mixtures comprising a flour according to the invention.

A further subject matter of the present invention is the use of genetically modified plant cells according to the invention, of plants according to the invention, of parts of plants according to the invention, of starch-storing parts of plants according to the invention, of propagation material according to the invention or of harvestable material according to the invention for the preparation of flours.

The disclosure of all documents cited in the patent application is intended to be incorporated in the disclosure of the present description of the invention.

DESCRIPTION OF THE SEQUENCES

SEQ ID NO 1: Nucleic acid sequence coding for a protein with the activity of a glucan, water dikinase from *Solanum tuberosum*.

SEQ ID NO 2: Amino acid sequence of the protein encoded by SEQ ID NO 1 with the activity of a glucan, water dikinase from *Solanum tuberosum*.

SEQ ID NO 3: Nucleic acid sequence coding for a protein with the activity of a starch synthase II from *Triticum aestivum*.

SEQ ID NO 4: Amino acid sequence of the protein encoded by SEQ ID NO 3 with the activity of a starch synthase II from *Triticum aestivum*.

SEQ ID NO 5: Nucleic acid sequence coding for a protein with the activity of a starch synthase II from *Oryza sativa*.

SEQ ID NO 6: Amino acid sequence of the protein encoded by SEQ ID NO 5 with the activity of a starch synthase II from *Oryza sativa*.

SEQ ID NO 7: Nucleic acid sequence coding for a protein with the activity of a GBSS I from *Oryza sativa*.

SEQ ID NO 8: Amino acid sequence of the protein encoded by SEQ ID NO 7 with the activity of a GBSS I from *Oryza sativa*.

SEQ ID NO 9: Nucleic acid sequence coding for a protein with the activity of a GBSS I from *Triticum aestivum*.

SEQ ID NO 10: Amino acid sequence of the protein encoded by SEQ ID NO 9 with the activity of a GBSS I from *Triticum aestivum*.

SEQ ID NO 11: Nucleic acid sequence coding for a protein with the activity of a GBSS I from *Zea mays*.

SEQ ID NO 12: Amino acid sequence of the protein encoded by SEQ ID NO 11 with the activity of a GBSS I from *Zea mays*.

General Methods

In the following text, methods will be described which can be used for carrying out the methods/processes according to the invention. These methods are specific embodiments of the present invention, but do not limit the present invention to these methods. The skilled worker knows that he can carry out the invention in the same manner by modifying the methods described and/or by replacing individual parts of the methods by alternative parts of methods. The content of all cited publications is incorporated into the description of the application by reference.

1. Transformation and Regeneration of Rice Plants

Rice plants were transformed by the method described by Hiei et al. (1994, *Plant Journal* 6(2), 271-282).

The regimen of the rice plants in the greenhouse involved the following conditions: sowing: substrate: mixture of 100% sphagnum peat and 100 l sand/m² and clay: 180 kg/m² in 1.6 l rose pots (manufacturer: H. Meyer, Germany), pH: 5.4-6.2; green manure: Hakaphos (Compo, Germany) 14% N-16% P-18% K+2% Mg; 2 kg/m²; fertilization: 3.5 g/plant until flowering: NH₄NO₃ (1.75 g) and Flory 2 basic mixture (manufacturer: Euflo, Germany): 1.75 g; 3% N-16% P-15% K+5% Mg.

Temperature: day 28° C./night: 24° C. (16 h/8 h); relative atmospheric humidity: 85-95%;

Light: 16 h, 350 μEinstein/sxm²

2. Origin of the Sequences and Constructs Used for the Transformation

The sequence T.a.-SSIIa from wheat was used for the transformation of rice. It was isolated and cloned as described in WO 97-45545 (under its then name "pTaSS1").

The transformation vector used, AH32-191, is described in example 2.

The sequence of a glucan, water dikinase from potato (R1St) was furthermore used. It was isolated and cloned as described in example 5. The transformation vector used, pML82, is described in WO 05/095619.

The waxy trait was introduced via a suitable mutant which is explained in example 1.

3. Analysis of the Expression Level of a Gene by Means of Northern Blot

The expression of a nucleic acid which codes for a protein was studied by means of Northern blot analysis. To this end, three immature rice grains (approximately 15 days after anthesis) were harvested for each individual plant obtained by means of transformation and frozen in liquid nitrogen. To homogenize the material, the frozen rice grains were comminuted for 30 seconds in a Retsch mill (model MM300) in a 96-well microtiter plate using a 4.5 mm steel ball at a frequency of 30 Hertz. Thereafter, the RNA was isolated by means of the Promega RNA extraction kit following the manufacturer's instructions (SV 96 Total RNA Isolation System, Order No. Z3505, Promega, Mannheim). The concentration of the RNA in the individual samples was determined by photometrically measuring the absorption at 260 nm.

For each sample, 2 μg of RNA were brought to a uniform volume and treated with an identical volume of RNA sample buffer (65% (v/v) formamide, 8% formaldehyde, 13% (v/v) gel buffer (see above), 50 μg/ml ethidium bromide). After heating (10 min, 65° C.) and immediate cooling on ice, the RNA was separated for approximately 2 hours using a 1.2% (w/v) agarose gel (20 mM MOPS pH 8.0, 5 mM sodium acetate, 1 mM EDTA, 6% (v/v) formaldehyde) using RNA running buffer (20 mM MOPS pH 8.0, 5 mM sodium acetate, 1 mM EDTA) at a constant amperage of 50-80 mA.

Thereafter, the RNA was transferred to a Hybond N membrane by means of diffusion blot using 10×SSC (1.5 M NaCl, 150 mM sodium citrate pH 7.0) and immobilized on the membrane by means of UV irradiation.

The hybridization of the Northern blot for detecting the expression of a nucleic acid molecule which codes for a protein with the activity of a starch synthase II from wheat employed an approx. 1 kb SpeI/BspHI fragment of the plasmid AH32-191 (bp 4568-5686), which encompasses the 5' region of the cDNA. The DNA fragment was radiolabeled by means of the Random Primed DNA Labeling Kit from Roche (Order No. 1004 760) using ³²P-alpha-dCTP and following the manufacturer's instructions. The nylon membrane com-

prising the transferred RNA was incubated for 4 hours at 60° C. in a water bath with hybridization buffer (250 mM sodium phosphate buffer pH 7.2, 1 mM EDTA, 6% (w/v) SDS, 1% (w/v) BSA), with gentle shaking, whereupon the radiolabel DNA was added to the hybridization buffer. After incubation for 16 hours, the hybridization buffer was removed, and the membrane was washed in succession once with 3×SSC and once with 2×SSC (see above) at 60° C., with gentle shaking, to remove unspecifically bound DNA molecules.

To detect labeled RNA, the nylon membrane was autoradiographed for one to three days at -70° C. on an x-ray film.

4. Determination of the Activity of a Protein with the Activity of a Starch Synthase II by Means of Activity Gels (Zymogram)

The detection of the activity of proteins with the activity of a starch synthase in immature rice grains was performed by means of activity gels (zymogramms), in which protein extracts are separated in a polyacrylamide gel under native conditions and subsequently incubated with suitable substrates. The reaction product formed (alpha-glucan) was stained in the gel using Lugol's solution.

Individual immature rice grains (approx. 15 days after anthesis) were frozen in liquid nitrogen and homogenized in 150-200 μl of cold extraction buffer (50 mM Tris/HCl pH 7.6, 2.5 mM EDTA, 2 mM DTT, 4 mM PMSF, 0.1% (w/v) glycogen, 10% (v/v) glycerol). After centrifugation (15 min, 13000 g, 4° C.), the clear supernatant was transferred into a fresh reaction vessel, and an aliquot of the extract was used for determining the protein content by the method of Bradford (1976, *Anal Biochem* 72: 248-254).

The protein extracts were separated by means of continuous 7.5% strength polyacrylamide gel (7.5% acrylamide: bisacrylamide 37.5:1; 25 mM Tris/HCl pH 7.6, 192 mM glycine, 0.1% (w/v) APS, 0.05% (v/v) TEMED) using running buffer in single concentration (25 mM Tris/HCl, 192 mM glycine). For each sample, amounts corresponding to 15 μg of protein were applied in each case, and the electrophoresis was run for 2 to 2.5 hours at 4° C.

Thereafter, the gels were incubated overnight at room temperature in 15 ml of incubation buffer (0.5 mM sodium citrate pH 7.0, 25 mM potassium acetate, 2 mM EDTA, 2 mM DTT, 0.1% (w/v) amylopectin, 50 mM tricine/NaOH pH 8.5, 1 mM ADP-glucose), with constant shaking. The starch formed was stained by means of Lugol's solution.

To determine by how many times the activity of a protein with the activity of a starch synthase II is increased in comparison with corresponding not genetically modified wild-type plants, protein extracts from the genetically modified lines were in each case subjected to sequential dilution and separated by electrophoresis in accordance with the above-described method. The remaining steps were carried out as already described above. After the zymogramms had been stained with Lugol's solution, the intensity of the stained products produced by a protein with the activity of a starch synthase II (identified by an arrow in FIG. 1) for the different dilutions of the protein extracts from genetically modified plants were compared visually with the relevant products of the undiluted wild-type protein extract. Since the intensity of the coloration of the products correlates directly with the activity of a protein with the activity of a starch synthase II, product bands with the same intensities have the same activity. If the band of the product of a protein with the activity of a starch synthase II in the dilute protein extract has the same intensity as the corresponding band of the product from corresponding undiluted protein extract from wild-type plants, the dilution factor corresponds to the degree of the increase in

the activity in the corresponding genetically modified plant (for comparisons, see FIG. 1).

5. Generation of Plants from Isolated Rice Embryos (Embryo Rescue)

Seeds are removed from the panicle, and the shells are removed. The endosperm is dissected from the embryo using a surgical blade and used for suitable analyses. To improve the wettability, the embryo is briefly treated with 70% ethanol and subsequently incubated for 20 minutes in a solution comprising 2% NaOCl and one drop of commercially available washing-up liquid to sterilize it.

Thereafter, as much as possible of the sterilization solution is removed, and the embryo is washed with sterile demineralized water, once for a minute and thereafter twice for in each case 10 minutes. The seeds are plated out in Petri dishes on agar solidified medium comprising in each case a quarter of the salt concentration of MS medium (Murashige-Skoog medium) and 4% sucrose. Thereafter, the Petri dishes are sealed using Parafilm and incubated in the dark at 23° C. After germination (approx. 5-7 days after plating out the embryos), the Petri dishes are transferred into the light. When the hypocotyls of the seedlings have reached a length of approx. 2 cm, the plants are transferred into jars comprising agar-solidified MS medium with 2% sucrose. After sufficient roots have developed, the plants can be potted in compost.

6. Processing of Rice Grains, and Preparation of Rice Flours

To prepare sufficient amounts of test material, rice plants were grown in the greenhouse and harvested when fully mature. The mature rice grains were stored for 3-7 days at 37° C. to dry them further.

Thereafter, the grains were freed from the shells by means of a sheller (Laboratory Paddy sheller, Grainman, Miami, Fla., USA), and the brown rice obtained was processed by polishing for 1 minute (Pearlest Rice Polisher, Kett, Villa Park, Calif., USA) to give white rice. For grain composition studies and starch property studies, the white grains were ground by means of a laboratory mill (Cyclotec, Sample mill, Foss, Denmark) to give what is known as rice flour.

7. Extraction of Rice Starch from Rice Flour

Rice starch was extracted from rice flour by a method similar to the method described by Wang and Wang (2004; Journal of Cereal Science 39: 291-296).

Approx. 10 g of rice flour were incubated for 16-18 hours with 40 ml of 0.05% (w/v) NaOH at room temperature on a shaker. Thereafter, the suspension was transferred into a Waring blender to complete the digestion and mixed for 15 seconds at low speed and subsequently for 45 seconds at high speed. To remove coarse constituents (for example cell wall), the suspension was poured in succession through sieves with a mesh size of 125 µm and of 63 µm. After centrifugation at 1500 rpm for 15 minutes (Microfuge 3.OR; Heraeus), the supernatant was decanted off, and the protein layer at the top of the sediment was removed using a spatula. The remainder of the sediment was resuspended in 0.05% (w/v) NaOH, and the procedure described above was repeated. Thereafter, the sediment was resuspended in water and the pH of the suspension was brought to 6.5 to 7 using HCl. The rice starch obtained was washed in total three times with water, where each wash step comprised a sedimentation (centrifugation at 1500 rpm, 15 min, RT), discarding the supernatant and resuspending the sediment in fresh water. Before the last wash step, the pH was rechecked and, if necessary, brought to pH 7 with HCl. The sediment of the last wash step was resuspended in acetone, sedimented and the supernatant was discarded. After resuspending the sediment again in acetone, the suspension was poured into a Petri dish and dried in a fume hood at room temperature for at least 18 hours.

In a last step, the resulting rice starch was made into a fine powder by comminuting in a pestel and mortar, and this powder can be employed directly for further studies.

8. Determination of the Hot-Water Swelling Power (SP)

100 mg of sample (starch or flour) are suspended in 10 ml of water and subsequently swelled for 20 minutes at 92.5° C. During the incubation of the sample of 92.5° C., the suspension is mixed repeatedly (continuously during the first 2 minutes, then after 3, 4, 5, 10, 15 and 25 minutes) by carefully turning the sample containers by 360°. After incubation for a total of 30 minutes at 92.5° C., the suspension is cooled for approx. 1 minute in ice-water before carrying out an incubation at 25° C. for 5 minutes. After centrifugation (room temperature, 1000×g, 15 minutes), the supernatant obtained is removed carefully from the gel-like sediment and the sediment weight is determined. The hot-water swelling power is calculated using the following formula:

$$SP = \frac{\text{weight of the gel-like sediments}}{\text{weight of the weighed-in sample (flour or starch)}}$$

9. Determination of the Starch Phosphate Content in the C6 Position of the Glucose Molecules

In starch, the positions C2, C3 and C6 of the glucose units may be phosphorylated. To determine the C6-P content of the starch or the flour (modified method of Nielsen et al., 1994, Plant Physiol. 105: 111-117), 50 mg of rice flour or rice starch were hydrolyzed for 4 hours in 500 µl of 0.7 M HCl at 95° C., with continuous shaking. Thereafter, the mixtures were centrifuged for 10 minutes at 15.500×g, and the supernatants were freed from suspended matter and cloudiness by means of a filter membrane (0.45 µm). 20 µl of the clear hydrolyzate were mixed with 180 µl of imidazol buffer (300 mM imidazol, pH 7.4; 7.5 mM MgCl₂, 1 mM EDTA and 0.4 mM NADP), and the samples were measured in a photometer at 340 nm. After recording the basic absorption, an enzyme reaction was started by addition of 2 units of glucose 6-phosphate dehydrogenase (from *Leuconostoc mesenteroides*, Boehringer Mannheim). The measured change (OD) is based on an equimolar conversion of glucose 6-phosphate and NADP to give 6-phosphogluconate and NADPH, where the formation of NADPH is recorded at the abovementioned wavelength. The reaction was monitored until an end point had been reached. The result of this measurement can be used for calculating the glucose 6-phosphate content in the hydrolyzate:

$$\text{nmol glucose 6-phosphate/mg FW} = \frac{OD \times \text{measuring volume} (200 \mu\text{l}) \times \text{hydrolyzate volume} (500 \mu\text{l})}{\text{extinction coefficient} \times \text{sample volume} (20 \mu\text{l}) \times \text{mg material weighed in} (50 \text{ mg})}$$

To avoid erroneous results caused by incomplete hydrolysis of the starch in the material weighed in (flour or starch), the degree of hydrolysis was subsequently determined. To this end, 10 µl of hydrolyzate was removed from the respective hydrolyzates which were measured by their glucose 6-phosphate content, neutralized with 10 µl of 0.7 M NaOH and brought to a final volume of 2 ml with water (dilution 1:200). 4 µl of this dilution were treated with 196 µl of measuring buffer (100 mM imidazole pH 6.9; 5 mM MgCl₂, 1 mM ATP, 0.4 mM NADP) and used for the photometric determination of the glucose content. After determining the basic absorption

at 340 nm, the reaction was monitored until the end point was reached in the photometer (340 nm) by addition of 2 μ l of enzyme mix (hexokinase 1:10; glucose 6-phosphate dehydrogenase from yeast 1:10 in measuring buffer). The principle of the measurement corresponds to that of the first reaction. Using the data obtained, the amount of glucose can be calculated for the sample in question:

$$\text{mmol Glucose/g FW} = \frac{\text{OD} \times \text{measuring volume} \times \text{hydrolyzate volume (500 } \mu\text{l)} \times \text{total volume of the dilution (2 ml)}}{\text{extinction coefficient} \times \text{sample volume (20 } \mu\text{l)} \times \text{volume employed for the dilution (10 } \mu\text{l)} \times \text{mg material weighed in (50 mg)}}$$

The amount of glucose detected in the individual samples corresponds to the amount of starch which is available for the C6-phosphate determination. To simplify the further calculation, the glucose content is converted into starch content.

$$\text{starch content (\%)} = \frac{\text{glucose content (mmol/g FW)} \times \text{molecular weight of glucose in starch (162 g/mol)} \times \text{conversion factor (\% = 100)}}{\text{conversion factor (mmol to mol = 1000)}}$$

In what follows, the result of the glucose 6-phosphate measurement is related to the starch content of the sample in question in order to express, in this manner, the glucose 6-phosphate content per mg of hydrolyzed starch:

$$\text{nmol Glc-6 P/mg starch} = \frac{\text{nmol glucose 6-phosphate/mg material weighed in} \times \text{starch content}}{\text{(mg starch/100 mg material weighed in)}}$$

In contrast to when relating the amount of glucose 6-phosphate to the weighed-in weight of the sample (flour or starch), this type of calculation relates the amount of glucose 6-phosphate only to the amount of starch which has been completely hydrolyzed to give glucose.

10. Determination of the Apparent Amylose Content

The determination of the apparent amylose content was carried out by a method similar to that of Juliano (1971, *Cereal Science Today* 16 (10): 334-340).

For each sample, 50 mg of rice flour were weighed, in duplicate, in 100 ml Erlenmeyer flasks and consecutively moistened with 1 ml of 95% strength ethanol and 9 ml of 1M NaOH.

In parallel, flasks with defined amounts of pure amylose from potato starch are treated in the same manner as the flour samples, in order to establish a calibration curve. The flasks were swirled briefly to mix the contents and subsequently incubated for 20 minutes in a boiling water bath, with gentle

shaking. After 5-10 minutes cooling at RT, the volume was made up to 100 ml with water.

A 100 μ l aliquot was treated with 1 ml measuring solution (10 mM acetic acid, 0.004% (w/v) I_2 ; 0.04% (w/v) KI), mixed thoroughly, and the absorption was determined at 620 nm against a suitable blank. The calculation of the amylose content was carried out with the aid of the amylose standards used for establishing a calibration curve.

11. Quantitative PCR

RNA was prepared from individual immature rice seeds (10-12 days after anthesis). After the seeds, which had been frozen in liquid nitrogen, had been homogenized using a 4 mm steel ball (Retsch mill, 30 Hz, 45 sec), the RNA was prepared using the "SV 96 Total RNA Isolation System" by Promega, following protocol No. 294 (Promega). The RNA was treated with in each case 10 μ l of "RQ1 RNase-Free DNase" (Promega), following the manufacturer's instructions.

Identical amounts of RNA from in each case four seeds of one plant were combined. The quantitative RT-PCR was carried out with reagents of the "Access RT-PCR System" by Promega.

The reaction conditions for the RT-PCR were: 30 min at 55° C., 2 min at 94° C., 40x(15 sec 94° C., 1 min 60° C.). The fluorescent signal was recorded using an ABI Prism 7700 apparatus (Applied Biosystems), in each case during the combined annealing/extension phase.

The controls which were employed in this approach were in each case mixtures without reverse transcriptases.

The relative expression was calculated as described by M. W. Pfaffl (2001, A new mathematical model for relative quantification in real-time RT-PCR, *Nucleic Acids Research* 29, No 9 00).

Examples

1. Generation and Selection of the Waxy (GBSSI Knock Out) Mutant

The waxy mutant originated from an agrobacteria-mediated transformation of rice. An analysis of the progeny revealed that the waxy phenotype of the rice grains is inherited independently of the phosphinotricine resistance introduced with the transformation. A sequence analysis of the GBSSI (waxy) gene revealed that the manifestation of the waxy phenotype gene can be attributed to the exchange of two nucleotides, as a result of which a premature stop codon is generated, which leads to a truncated and probably inactive protein. The RFLP analysis of the apparent amylose content of the starch present in the rice grains confirmed a value of less than 5% by weight, which means that the mutant identified is a "waxy" mutant. As a consequence, the term "waxy phenotype" is understood as meaning waxy mutants whose starch has an apparent amylose content of less than 5%.

Lines 738-104 and 738-106, which are homozygous for the above-mentioned mutation, were used for the combination with the transgenic approaches.

		BamHI
M202		GAG TGG GAT CCT AGC
Waxy_Mutant		GAG TGA AAT CCT AGC
		Stop

2. Preparation of the Plant Expression Vector pAH32-191, which Comprises a Coding Sequence for a Protein with the Activity of a Starch Synthase II

The complete encoding sequence of the protein with the activity of a starch synthase II from wheat (T.a.-SSII) was excised from the plasmid pCF31 (described in WO 97/45545 under the name pTaSS1) by means of the restriction endonucleases Ecl13611 and Xho I and cloned into the plasmid pIR103-123 (described in WO 05/030941) which had been cleaved with the restriction endonucleases Eco RV and Xho I. The expression vector obtained was named pAH32-191. The plant expression vector pIR103-123 serves for the endosperm-specific expression of the target gene under the control of the endosperm-specific globulin promoter (Nakase et al. (1996) Gene 170(2): 223-226) from rice. In addition, the plant expression vector pIR103-123 comprises the bar gene under the control of the CaMV 35S promoter, which gene was used as the selection marker for the transformation of plants.

3. Generation of Rice Plants with an Increased Activity of a Protein with the Activity of a Starch Synthase II

Rice plants (variety M202) were transformed by means of agrobacteria comprising the plasmid pAH32-191 using the method described by Hiei et al. (1994, Plant Journal 6(2), 271-282). The resulting plants were named oe-SSII-O.s.-X, where X means independent plants obtained from the transformation.

4. Analysis of the Rice Plants which Had been Transformed with the Expression Vector pAH32-191

Rice plants (T0 plants) of the lines named oe-SSII-O.s.-X and which had originated from the transformation with the expression vector pAH32-191 were grown in soil in the greenhouse. RNA was isolated from immature grains (T1 seeds) of various lines, and a Northern blot analysis was carried out in accordance with the method described in "General Methods", using an SSII-specific probe. A plurality of lines with an increased amount of transcript of the wheat starch synthase II in comparison with corresponding not genetically modified wild-type plants were identified (see diagram shown by way of example in FIG. 2).

In addition, an increased activity of a protein with the activity of a starch synthase II in protein extracts of immature T1 seeds from different lines of the above-mentioned transformation was determined by means of zymograms (see diagram shown by way of example in FIGS. 1 and 2). The analysis was carried out by means of zymograms as described in "General Methods".

Based on the results of the analyses described, the following line was selected for the combination with other approaches:

oe-SSII-O.s-01502

On the basis of a variety of analyses, it was possible to demonstrate that this line is homozygous for the integrations of the T-DNA(s) of the vector pAH32-191.

5. Generation of Rice Plants with an Increased Activity of a Protein with the Activity of a Glucan, Water Dikinase

Rice plants (variety M202) were transformed by means of agrobacteria which comprise the plasmid pML82 (described in WO 05/095619), using the method described by Hiei et al. (1994, Plant Journal 6(2), 271-282). The resulting plants were named oe-GWD-O.s.-X, where X means independent plants obtained from the transformation.

6. Analysis of the Rice Plants which Had been Transformed with the Expression Vector pML82

Rice plants (T0 plants) of the lines named oe-GWD-O.s.-X and which had originated from the transformation with the expression vector pML82 were grown in soil in the greenhouse. Individual, mature grains (T1 seeds) from different lines were made into a flour. To this end, individual grains were comminuted, in a ball mill (from Retsch, Model MM300), for 30 seconds at a frequency of 30 Hertz in an Eppendorf reaction vessel using a tungsten carbide ball. This was followed by a determination of the starch phosphate content in the C6 position of glucose molecules of the starch present in the flour as described in "General Methods".

The following results were obtained for selected plants:

TABLE 1

Starch phosphate content in the C6 position of the glucose molecules of individual T1 seeds from different lines with the name oe-GWD-O.s.-X in comparison with seeds of corresponding not genetically modified wild-type plants (WT) of variety M202.	
Line	nmol C6P/mg material weighed
oe-GWD-O.s.-2	1.68
oe-GWD-O.s.-4	1.70
oe-GWD-O.s.-9	1.47
WT	0.30

As can be seen from table 1, it was possible to identify independent lines which are the result of the transformation with the plant expression vector pML82 and which, in comparison with corresponding not genetically modified wild-type plants have an increased starch phosphate content in the C6 position of the glucose molecules. It is known that plant cells with an increased expression of a protein with the activity of a glucan, water dikinase synthesize a starch with a higher starch phosphate content in comparison with corresponding genetically not modified wild-type plants (see, for example, WO 02/34923).

Based on the above-described analyses, the following lines were selected for the combination with other approaches:

oe-GWD-O.s.-2

oe-GWD-O.s.-4

oe-GWD-O.s.-9

On the basis of various analyses, it was possible to demonstrate that these lines are homozygous for the integrations of the T-DNA(s) of vector pML82.

47

7. Generation of Plants with a Waxy Phenotype and an Increased Activity of a Protein with the Activity of a Glucan, Water Dikinase

The following crosses were made:

TABLE 2

Crosses of the combination of 738-104/4 (M202 waxy) with oe-GWD-O.s.				
Pedigree cross	Name of female parent	Plasmid of female parent	Name of male parent	Plasmid of male parent
XPOS0001	M202 waxy	—	oe-GWD-O.s.	pML82
-01	738-106	—	oe-GWD-O.s-2	pML82
-02	738-104	—	oe-GWD-O.s-2	pML82
-03	738-104	—	oe-GWD-O.s-4	pML82
-04	738-106	—	oe-GWD-O.s-4	pML82
-05	738-104	—	oe-GWD-O.s-9	pML82
-06	738-106	—	oe-GWD-O.s-9	pML82

The endosperm of the F1 seeds, which were the result of the cross, was studied for the starch phosphate content in the C6 position of the glucose molecules (C6P). The embryos of those grains whose starch phosphate content (C6P) was markedly increased in comparison with the female parent were germinated by means of tissue culture techniques. After a sufficient size had been attained, relevant plants were transferred to the greenhouse in order to produce F2 seeds.

Grains with waxy phenotype were selected from the mature F2 seeds by means of visual scoring and placed in the greenhouse. After germination, the plants were sprayed with Basta® (Bayer CropScience), and leaf samples were taken from Basta®-tolerant plants. Plants which were homozygous for the integration of the T-DNA of vector pML82 were identified by means of a copy number determination using invader technology (http://www.twt.com/invader_chemistry/invaderchem.htm; Ledford et al (2000, J. of Mol. Diagnostics. 2(2): 97-104; Mein et al., 2000, Genome Res. 10: 330-343) for the bar gene. The plants thus selected were grown on in the greenhouse for the production of F3 seeds.

Some mature F3 seeds of the potentially doubly homozygous plants were studied individually for their starch phosphate (C6P) content. Those plants where all grains had an expectedly high starch phosphate (C6P) content were retained.

The seed of all doubly homozygous plants of a parental combination was pooled and used for further propagation and for grain and flour property analyses.

For the combination with line oe-SSII-O.s, the event XPOS0001-05, which is homozygous both for the waxy mutation and for the T-DNA of the vector pML82, was selected.

48

8. Generation of Plants with a Waxy Phenotype and with an Increased Activity of a Protein with the Activity of a Glucan, Water Dikinase and with an Increased Activity of a Protein with the Activity of a Starch Synthase II

The following crosses were made:

TABLE 3

Crosses of the combination of oe-SSII-O.s. with XPOS0001-05				
Pedigree cross	Female parent	Plasmid of female parent	Male parent	Plasmid of male parent
XPOS0025-01	oe-SSII-O.s.-01502	pAH31-191	XPOS0001-05	pML82
XPOS0026-01	XPOS0001-05	pML82	oe-SSII-O.s.-01502	pAH32-191

Successful events in crosses were identified by measuring the starch phosphate content of the F1 endosperm, since the starch phosphate content of the combination is markedly higher than that of the parental lines.

9. Analysis of Plants with a Waxy Phenotype and with an Increased Activity of a Protein with the Activity of a Glucan, Water Dikinase and with an Increased Activity of a Protein with the Activity of a Starch Synthase II

Embryos of F1 seeds whose endosperm has a starch phosphate content of more than 5 nmol C6P/mg starch and is therefore markedly above that of both parents (2.5 nmol/mg starch for oe-GWD-O.s. and at least 0.8 nmol/mg starch for oe-SSII-O.s.) were germinated by means of tissue culture techniques, and the plants in question, once they had reached a suitable size, were transferred to the greenhouse to produce F2 seeds.

To identify progeny which is homozygous for both transgenes and for the waxy mutation, the above-described procedure was repeated for F2 seeds which had been preselected visually with regard to a “waxy phenotype”, including the embryo rescue.

10. Selection and Analysis of the F2 Plants

Based on the results of the starch phosphate measurement, F2 seeds were selected (C6P>8 nmol/mg starch), their embryos were germinated, and the F2 plants in question were grown in the greenhouse.

Genomic DNA was extracted from leaf material of the F2 plants, and the copy number of the two transgenes and of the bar gene (total of the values for the two transgenes) was determined by means of quantitative PCR.

The proof that the waxy mutation was homozygous was carried out using an RFLP(Bam HI) in the GBSSI gene (definition and/or method) of the waxy mutant. F2 plants which are potentially homozygous for the two transgenes and homozygous for the waxy RFLP were grown on in the greenhouse and used for the production of F3 seeds.

11. Selection of the F3 Plants/Analysis of F3 Seeds

To identify triply homozygous lines, some individual grains of suitably selected plants were examined visually for a waxy phenotype and subsequently studied for their starch phosphate content. If all grains have a waxy phenotype, and if the starch phosphate content for all grains of one plant is found to be approximately equally high, it can be assumed

that the plant is homozygous for the waxy mutation and for the T-DNA of pML82 and pAH32-191.

12. Generation of F4 Material

The following lines were found in the abovementioned analysis to be triply homozygous:

- XPOS002501-1-37
- XPOS002501-1-13
- XPOS002601-1-19

Plants from these lines were grown in the greenhouse, and the F4 seeds produced were harvested and dried and then pooled as one line for all progeny.

13. Functionalities and Analysis of the Constituents of the F4 Material

a) Grain Composition

Apparent Amylose Content:

TABLE 4

Apparent amylose content in rice flours and rice starches for the single-gene approaches and the triple combination		
Sample name	Apparent amylose content of rice flours (% amylose/FW)	Apparent amylose content of rice starches (% amylose/FW)
Wild type	8.9	11.8
oe-GWD-O.s.-4	10.6	14.4
oe-GWD-O.s.-9	10.6	14.3
oe-SSII-O.s.-01502	6.6	9.2
738-104/6	2.3	2.2
XPOS025-01-1-37	3.7	3.5
XPOS025-01-1-13	3.7	3.7
XPOS026-01-1-19	3.9	4.1

It emerged that the combinations XPOS0025/6 have an amylose content above that of the waxy mutant (738-104/6).
Starch Phosphate Content (C6P Contents)

TABLE 5

Starch phosphate content in the C6 position of rice flours or starches for the single-gene approaches and for the triple combinations		
Sample name	Starch phosphate content in the C6 position of starches present in rice flours (nmol C6P/mg starch)	Starch phosphate content in the C6 position of rice starches (nmol C6P/mg starch)
Wild type	0.46	0.37
oe-GWD-O.s.-4	2.85	2.65
oe-GWD-O.s.-9	3.27	2.56
oe-SSII-O.s.-01502	1.22	0.91
738-104/6	0.52	0.38
XPOS025-01-1-37	11.45	9.50
XPOS025-01-1-13	11.20	10.24
XPOS026-01-1-19	11.06	10.23

The starch phosphate content in the C6 position of the triple combination is markedly higher than that of the single-gene approaches.

b) Functionalities of Rice Flours and Rice Starches Hot-Water Swelling Power

TABLE 6

Hot-water swelling power of rice flours or rice starches of the single-gene approaches and of the triple combination		
Sample name	Hot-water swelling power of rice flours (g/g)	Hot-water swelling power of rice starches (g/g)
Wild type	15.7	31.9
oe-GWD-O.s.-4	21.6	38.6
oe-GWD-O.s.-9	21.3	39.9
oe-SSII-O.s.-01502	20.2	40.8
738-104/6	19.9	47.3
XPOS025-01-1-37	40.6	86.0
XPOS025-01-1-13	41.9	89.1
XPOS026-01-1-19	38.3	87.2

The determination of the hot-water swelling power of flours or starches prepared from F4 seeds of the abovementioned lines and from wild-type plants was accomplished as described in "General Method".

The hot-water swelling power of the triple combination is markedly above that of the single-gene approaches.

DESCRIPTION OF THE FIGURES

FIG. 1 shows zymograms for determining the activity of proteins with the activity of a starch synthase II in comparison with the wild type. The material used were total protein extracts from immature grains (15 days after anthesis) of wild-type plants (WT) and of the three independent genetically modified plants which are the result of the transformations with the expression vector AH32-191 (oe-SSII-O.s.-5, oe-SSII-O.s.-12, oe-SSII-O.s.-19). In the lanes WT and pur, in each case identical amounts of protein of the respective extracts are applied. The protein extracts of the genetically modified plants were subjected to serial dilution (1:2, 1:4, 1:6, 1:8, 1:10, 1:20, 1:50 or 1:100), and these dilutions were separated by electrophoresis, also separately from one another. The increase in the activity of a starch synthase II in comparison with wild-type plants can be determined by comparing the intensity of the specific products which are present in the zymogram after staining with Lugol's solution and which have been synthesized by a protein with the activity of a starch synthase II (identified by an arrow) of protein extracts from wild-type plants with the intensity of the corresponding bands of protein extracts from genetically modified plants. Equal intensities mean equal activities.

FIG. 2 shows the autoradiogram of a Northern blot analysis of immature T1 seeds of the rice lines oe-SSII-O.s.-19, oe-SSII-O.s.-20, oe-SSII-O.s.-21, oe-SSII-O.s.-22, oe-SSII-O.s.-23 in comparison with not genetically modified wild-type plants (WT). To this end, RNA was extracted from in each case three seeds of lines which have independently originated from the transformation with the expression vector AH32-191 and was analyzed in accordance with the method described in General Methods, item 8. The band which hybridizes with a labeled nucleic acid probe coding for a protein with the activity of a starch synthase II from wheat is identified as SSII.

FIG. 3 shows a zymogram of protein extracts from immature T1 seeds of the rice lines oe-SSII-O.s.-8, oe-SSII-O.s.-19, oe-SSII-O.s.-23 in comparison with seeds of not genetically modified wild-type plants (WT) after staining with Lugol's solution. Protein extracts from two (oe-SSII-O.s.-8)

or three (oe-SSII-O.s.-19, oe-SSII-O.s.-23) different grains were analyzed per line. The analysis by means of zymogram was performed following the method described in General

Methods, item 9. The band in the zymogram which is specific for a protein with the activity of a starch synthase II is identified as SSII.

SEQUENCE LISTING

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<300> PUBLICATION INFORMATION:

<308> DATABASE ACCESSION NUMBER: EMBL / Y09533

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Tyr Asp Arg Ala Ile His Ser Glu Pro Asn Phe Arg Gly Asp Gln Lys	
745 750 755	
ggt ggt ctt ttg cgt gat tta ggt cac tat atg aga aca ttg aag gca	2420
Gly Gly Leu Leu Arg Asp Leu Gly His Tyr Met Arg Thr Leu Lys Ala	
760 765 770	
gtt cat tca ggt gca gat ctt gag tct gct att gca aac tgc atg ggc	2468
Val His Ser Gly Ala Asp Leu Glu Ser Ala Ile Ala Asn Cys Met Gly	
775 780 785	
tac aaa act gag gga gaa ggc ttt atg gtt gga gtc cag ata aat cct	2516
Tyr Lys Thr Glu Gly Glu Gly Phe Met Val Gly Val Gln Ile Asn Pro	
790 795 800	
gta tca ggc ttg cca tct ggc ttt cag gac ctc ctc cat ttt gtc tta	2564
Val Ser Gly Leu Pro Ser Gly Phe Gln Asp Leu Leu His Phe Val Leu	
805 810 815 820	
gac cat gtg gaa gat aaa aat gtg gaa act ctt ctt gag aga ttg cta	2612
Asp His Val Glu Asp Lys Asn Val Glu Thr Leu Leu Glu Arg Leu Leu	
825 830 835	

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gag gct cgt gag gag ctt agg ccc ttg ctt ctc aaa cca aac aac cgt Glu Ala Arg Glu Glu Leu Arg Pro Leu Leu Leu Lys Pro Asn Asn Arg 840 845 850	2660
cta aag gat ctg ctg ttt ttg gac ata gca ctt gat tct aca gtt aga Leu Lys Asp Leu Leu Phe Leu Asp Ile Ala Leu Asp Ser Thr Val Arg 855 860 865	2708
aca gca gta gaa agg gga tat gaa gaa ttg aac aac gct aat cct gag Thr Ala Val Glu Arg Gly Tyr Glu Glu Leu Asn Asn Ala Asn Pro Glu 870 875 880	2756
aaa atc atg tac ttc atc tcc ctc gtt ctt gaa aat ctc gca ctc tct Lys Ile Met Tyr Phe Ile Ser Leu Val Leu Glu Asn Leu Ala Leu Ser 885 890 895 900	2804
gtg gac gat aat gaa gat ctt gtt tat tgc ttg aag gga tgg aat caa Val Asp Asp Asn Glu Asp Leu Val Tyr Cys Leu Lys Gly Trp Asn Gln 905 910 915	2852
gct ctt tca atg tcc aat ggt ggg gac aac cat tgg gct tta ttt gca Ala Leu Ser Met Ser Asn Gly Gly Asp Asn His Trp Ala Leu Phe Ala 920 925 930	2900
aaa gct gtg ctt gac aga acc cgt ctt gca ctt gca agc aag gca gag Lys Ala Val Leu Asp Arg Thr Arg Leu Ala Leu Ala Ser Lys Ala Glu 935 940 945	2948
tgg tac cat cac tta ttg cag cca tct gcc gaa tat cta gga tca ata Trp Tyr His His Leu Leu Gln Pro Ser Ala Glu Tyr Leu Gly Ser Ile 950 955 960	2996
ctt ggg gtg gac caa tgg gct ttg aac ata ttt act gaa gaa att ata Leu Gly Val Asp Gln Trp Ala Leu Asn Ile Phe Thr Glu Glu Ile Ile 965 970 975 980	3044
cgt gct gga tca gca gct tca tta tcc tct ctt ctt aat aga ctc gat Arg Ala Gly Ser Ala Ala Ser Leu Ser Ser Leu Leu Asn Arg Leu Asp 985 990 995	3092
ccc gtg ctt cgg aaa act gca aat cta gga agt tgg cag att atc Pro Val Leu Arg Lys Thr Ala Asn Leu Gly Ser Trp Gln Ile Ile 1000 1005 1010	3137
agt cca gtt gaa gcc gtt gga tat gtt gtc gtt gtg gat gag ttg Ser Pro Val Glu Ala Val Gly Tyr Val Val Val Val Asp Glu Leu 1015 1020 1025	3182
ctt tca gtt cag aat gaa atc tac gag aag ccc acg atc tta gta Leu Ser Val Gln Asn Glu Ile Tyr Glu Lys Pro Thr Ile Leu Val 1030 1035 1040	3227
gca aaa tct gtt aaa gga gag gag gaa att cct gat ggt gct gtt Ala Lys Ser Val Lys Gly Glu Glu Glu Ile Pro Asp Gly Ala Val 1045 1050 1055	3272
gcc ctg ata aca cca gac atg cca gat gtt ctt tca cat gtt tct Ala Leu Ile Thr Pro Asp Met Pro Asp Val Leu Ser His Val Ser 1060 1065 1070	3317
gtt cga gct aga aat ggg aag gtt tgc ttt gct aca tgc ttt gat Val Arg Ala Arg Asn Gly Lys Val Cys Phe Ala Thr Cys Phe Asp 1075 1080 1085	3362
ccc aat ata ttg gct gac ctc caa gca aag gaa gga agg att ttg Pro Asn Ile Leu Ala Asp Leu Gln Ala Lys Glu Gly Arg Ile Leu 1090 1095 1100	3407
ctc tta aag cct aca cct tca gac ata atc tat agt gag gtg aat Leu Leu Lys Pro Thr Pro Ser Asp Ile Ile Tyr Ser Glu Val Asn 1105 1110 1115	3452
gag att gag ctc caa agt tca agt aac ttg gta gaa gct gaa act Glu Ile Glu Leu Gln Ser Ser Ser Asn Leu Val Glu Ala Glu Thr 1120 1125 1130	3497
tca gca aca ctt aga ttg gtg aaa aag caa ttt ggt ggt tgt tac Ser Ala Thr Leu Arg Leu Val Lys Lys Gln Phe Gly Gly Cys Tyr 1135 1140 1145	3542

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tca cgt aat att gca tat ctg aaa gga aaa gtg cct tcc tcg gtg	3632
Ser Arg Asn Ile Ala Tyr Leu Lys Gly Lys Val Pro Ser Ser Val	
1165 1170 1175	
gga att cct acg tca gta gct ctt cca ttt gga gtc ttt gag aaa	3677
Gly Ile Pro Thr Ser Val Ala Leu Pro Phe Gly Val Phe Glu Lys	
1180 1185 1190	
gta ctt tca gac gac ata aat cag gga gtg gca aaa gag ttg caa	3722
Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala Lys Glu Leu Gln	
1195 1200 1205	
att ctg atg aaa aaa cta tct gaa gga gac ttc agc gct ctt ggt	3767
Ile Leu Met Lys Lys Leu Ser Glu Gly Asp Phe Ser Ala Leu Gly	
1210 1215 1220	
gaa att cgc aca acg gtt tta gat ctt tca gca cca gct caa ttg	3812
Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Ala Pro Ala Gln Leu	
1225 1230 1235	
gtc aaa gag ctg aag gag aag atg cag ggt tct ggc atg cct tgg	3857
Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly Met Pro Trp	
1240 1245 1250	
cct ggt gat gaa ggt cca aag cgg tgg gaa caa gca tgg atg gcc	3902
Pro Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala Trp Met Ala	
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ata aaa aag gtg tgg gct tca aaa tgg aat gag aga gca tac ttc	3947
Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg Ala Tyr Phe	
1270 1275 1280	
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Ser Thr Arg Lys Val Lys Leu Asp His Asp Tyr Leu Cys Met Ala	
1285 1290 1295	
gtc ctt gtt caa gaa ata ata aat gct gat tat gca ttt gtc att	4037
Val Leu Val Gln Glu Ile Ile Asn Ala Asp Tyr Ala Phe Val Ile	
1300 1305 1310	
cac aca acc aac cca tct tcc gga gac gac tca gaa ata tat gcc	4082
His Thr Thr Asn Pro Ser Ser Gly Asp Asp Ser Glu Ile Tyr Ala	
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1330 1335 1340	
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Gly Arg Ala Leu Ser Phe Ile Cys Lys Lys Lys Asp Leu Asn Ser	
1345 1350 1355	
cct caa gtg tta ggt tac cca agc aaa ccg atc ggc ctt ttc ata	4217
Pro Gln Val Leu Gly Tyr Pro Ser Lys Pro Ile Gly Leu Phe Ile	
1360 1365 1370	
aaa aga tct atc atc ttc cga tct gat tcc aat ggg gaa gat ttg	4262
Lys Arg Ser Ile Ile Phe Arg Ser Asp Ser Asn Gly Glu Asp Leu	
1375 1380 1385	
gaa ggt tat gcc ggt gct ggc ctc tac gac agt gta cca atg gat	4307
Glu Gly Tyr Ala Gly Ala Gly Leu Tyr Asp Ser Val Pro Met Asp	
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gag gag gaa aaa gtt gta att gat tac tct tcc gac cca ttg ata	4352
Glu Glu Glu Lys Val Val Ile Asp Tyr Ser Ser Asp Pro Leu Ile	
1405 1410 1415	
act gat ggt aac ttc cgc cag aca atc ctg tcc aac att gct cgt	4397
Thr Asp Gly Asn Phe Arg Gln Thr Ile Leu Ser Asn Ile Ala Arg	
1420 1425 1430	
gct gga cat gct atc gag gag cta tat ggc tct cct caa gac att	4442
Ala Gly His Ala Ile Glu Glu Leu Tyr Gly Ser Pro Gln Asp Ile	
1435 1440 1445	

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gag ggt gta gtg  agg gat gga aag att  tat gtc gtt cag aca  aga      4487
Glu Gly Val Val  Arg Asp Gly Lys Ile  Tyr Val Val Gln Thr  Arg
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cca cag atg tga ttatattctc gttgtatggt gttcagagaa gaccacagat      4539
Pro Gln Met

gtgatcatat tctcattgta tcagatctgt gaccacttac ctgatacctc ccatgaagtt  4599

acctgtatga ttatacgtga tccaaagcca tcacatcatg ttcaccttca gctattggag  4659

gagaagtgag aagtaggaat tgcaatatga ggaataataa gaaaaacttt gtaaaagcta  4719

aattagctgg gtatgatata gggagaaatg tgtaaacatt gtactatata tagtatatac  4779

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<210> SEQ ID NO 2
<211> LENGTH: 1464
<212> TYPE: PRT
<213> ORGANISM: Solanum tuberosum

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<400> SEQUENCE: 2

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Gly Asn Ser Leu Phe Gln Gln Gln Val Ile Ser Lys Ser Pro Leu Ser
          35          40          45

Thr Glu Phe Arg Gly Asn Arg Leu Lys Val Gln Lys Lys Lys Ile Pro
 50          55          60

Met Glu Lys Lys Arg Ala Phe Ser Ser Ser Pro His Ala Val Leu Thr
 65          70          75          80

Thr Asp Thr Ser Ser Glu Leu Ala Glu Lys Phe Ser Leu Gly Gly Asn
          85          90          95

Ile Glu Leu Gln Val Asp Val Arg Pro Pro Thr Ser Gly Asp Val Ser
          100          105          110

Phe Val Asp Phe Gln Val Thr Asn Gly Ser Asp Lys Leu Phe Leu His
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Trp Gly Ala Val Lys Phe Gly Lys Glu Thr Trp Ser Leu Pro Asn Asp
          130          135          140

Arg Pro Asp Gly Thr Lys Val Tyr Lys Asn Lys Ala Leu Arg Thr Pro
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Phe Val Lys Ser Gly Ser Asn Ser Ile Leu Arg Leu Glu Ile Arg Asp
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Thr Ala Ile Glu Ala Ile Glu Phe Leu Ile Tyr Asp Glu Ala His Asp
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Lys Trp Ile Lys Asn Asn Gly Gly Asn Phe Arg Val Lys Leu Ser Arg
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Lys Glu Ile Arg Gly Pro Asp Val Ser Val Pro Glu Glu Leu Val Gln
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Ile Gln Ser Tyr Leu Arg Trp Glu Arg Lys Gly Lys Gln Asn Tyr Pro
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Pro Glu Lys Glu Lys Glu Glu Tyr Glu Ala Ala Arg Thr Val Leu Gln
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Glu Glu Ile Ala Arg Gly Ala Ser Ile Gln Asp Ile Arg Ala Arg Leu
          260          265          270

Thr Lys Thr Asn Asp Lys Ser Gln Ser Lys Glu Glu Pro Leu His Val

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290					295					300					
Arg	Trp	Glu	Lys	Ala	Gly	Lys	Pro	Asn	Tyr	Pro	Pro	Glu	Lys	Gln	Ile
305					310					315					320
Glu	Glu	Leu	Glu	Glu	Ala	Arg	Arg	Glu	Leu	Gln	Leu	Glu	Leu	Glu	Lys
				325					330					335	
Gly	Ile	Thr	Leu	Asp	Glu	Leu	Arg	Lys	Thr	Ile	Thr	Lys	Gly	Glu	Ile
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Lys	Thr	Lys	Val	Glu	Lys	His	Leu	Lys	Arg	Ser	Ser	Phe	Ala	Val	Glu
		355					360					365			
Arg	Ile	Gln	Arg	Lys	Lys	Arg	Asp	Phe	Gly	His	Leu	Ile	Asn	Lys	Tyr
	370					375					380				
Thr	Ser	Ser	Pro	Ala	Val	Gln	Val	Gln	Lys	Val	Leu	Glu	Glu	Pro	Pro
385						390					395				400
Ala	Leu	Ser	Lys	Ile	Lys	Leu	Tyr	Ala	Lys	Glu	Lys	Glu	Glu	Gln	Ile
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Asp	Asp	Pro	Ile	Leu	Asn	Lys	Lys	Ile	Phe	Lys	Val	Asp	Asp	Gly	Glu
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Leu	Leu	Val	Leu	Val	Ala	Lys	Ser	Ser	Gly	Lys	Thr	Lys	Val	His	Leu
		435					440						445		
Ala	Thr	Asp	Leu	Asn	Gln	Pro	Ile	Thr	Leu	His	Trp	Ala	Leu	Ser	Lys
						455					460				
Ser	Pro	Gly	Glu	Trp	Met	Val	Pro	Pro	Ser	Ser	Ile	Leu	Pro	Pro	Gly
465						470					475				480
Ser	Ile	Ile	Leu	Asp	Lys	Ala	Ala	Glu	Thr	Pro	Phe	Ser	Ala	Ser	Ser
				485					490					495	
Ser	Asp	Gly	Leu	Thr	Ser	Lys	Val	Gln	Ser	Leu	Asp	Ile	Val	Ile	Glu
			500					505					510		
Asp	Gly	Asn	Phe	Val	Gly	Met	Pro	Phe	Val	Leu	Leu	Ser	Gly	Glu	Lys
		515					520						525		
Trp	Ile	Lys	Asn	Gln	Gly	Ser	Asp	Phe	Tyr	Val	Gly	Phe	Ser	Ala	Ala
	530					535					540				
Ser	Lys	Leu	Ala	Leu	Lys	Ala	Ala	Gly	Asp	Gly	Ser	Gly	Thr	Ala	Lys
545						550					555				560
Ser	Leu	Leu	Asp	Lys	Ile	Ala	Asp	Met	Glu	Ser	Glu	Ala	Gln	Lys	Ser
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Phe	Met	His	Arg	Phe	Asn	Ile	Ala	Ala	Asp	Leu	Ile	Glu	Asp	Ala	Thr
			580					585					590		
Ser	Ala	Gly	Glu	Leu	Gly	Phe	Ala	Gly	Ile	Leu	Val	Trp	Met	Arg	Phe
		595					600					605			
Met	Ala	Thr	Arg	Gln	Leu	Ile	Trp	Asn	Lys	Asn	Tyr	Asn	Val	Lys	Pro
	610					615					620				
Arg	Glu	Ile	Ser	Lys	Ala	Gln	Asp	Arg	Leu	Thr	Asp	Leu	Leu	Gln	Asn
625						630					635				640
Ala	Phe	Thr	Ser	His	Pro	Gln	Tyr	Arg	Glu	Ile	Leu	Arg	Met	Ile	Met
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Ser	Thr	Val	Gly	Arg	Gly	Gly	Glu	Gly	Asp	Val	Gly	Gln	Arg	Ile	Arg
			660					665					670		
Asp	Glu	Ile	Leu	Val	Ile	Gln	Arg	Asn	Asn	Asp	Cys	Lys	Gly	Gly	Met
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Met	Gln	Glu	Trp	His	Gln	Lys	Leu	His	Asn	Asn	Thr	Ser	Pro	Asp	Asp
	690					695					700				

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Val	Val	Ile	Cys	Gln	Ala	Leu	Ile	Asp	Tyr	Ile	Lys	Ser	Asp	Phe	Asp	705	710	715	720
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Arg	Leu	Leu	Ser	Tyr	Asp	Arg	Ala	Ile	His	Ser	Glu	Pro	Asn	Phe	Arg	740	745	750	
Gly	Asp	Gln	Lys	Gly	Gly	Leu	Leu	Arg	Asp	Leu	Gly	His	Tyr	Met	Arg	755	760	765	
Thr	Leu	Lys	Ala	Val	His	Ser	Gly	Ala	Asp	Leu	Glu	Ser	Ala	Ile	Ala	770	775	780	
Asn	Cys	Met	Gly	Tyr	Lys	Thr	Glu	Gly	Glu	Gly	Phe	Met	Val	Gly	Val	785	790	795	800
Gln	Ile	Asn	Pro	Val	Ser	Gly	Leu	Pro	Ser	Gly	Phe	Gln	Asp	Leu	Leu	805	810	815	
His	Phe	Val	Leu	Asp	His	Val	Glu	Asp	Lys	Asn	Val	Glu	Thr	Leu	Leu	820	825	830	
Glu	Arg	Leu	Leu	Glu	Ala	Arg	Glu	Glu	Leu	Arg	Pro	Leu	Leu	Leu	Lys	835	840	845	
Pro	Asn	Asn	Arg	Leu	Lys	Asp	Leu	Leu	Phe	Leu	Asp	Ile	Ala	Leu	Asp	850	855	860	
Ser	Thr	Val	Arg	Thr	Ala	Val	Glu	Arg	Gly	Tyr	Glu	Glu	Leu	Asn	Asn	865	870	875	880
Ala	Asn	Pro	Glu	Lys	Ile	Met	Tyr	Phe	Ile	Ser	Leu	Val	Leu	Glu	Asn	885	890	895	
Leu	Ala	Leu	Ser	Val	Asp	Asp	Asn	Glu	Asp	Leu	Val	Tyr	Cys	Leu	Lys	900	905	910	
Gly	Trp	Asn	Gln	Ala	Leu	Ser	Met	Ser	Asn	Gly	Gly	Asp	Asn	His	Trp	915	920	925	
Ala	Leu	Phe	Ala	Lys	Ala	Val	Leu	Asp	Arg	Thr	Arg	Leu	Ala	Leu	Ala	930	935	940	
Ser	Lys	Ala	Glu	Trp	Tyr	His	His	Leu	Leu	Gln	Pro	Ser	Ala	Glu	Tyr	945	950	955	960
Leu	Gly	Ser	Ile	Leu	Gly	Val	Asp	Gln	Trp	Ala	Leu	Asn	Ile	Phe	Thr	965	970	975	
Glu	Glu	Ile	Ile	Arg	Ala	Gly	Ser	Ala	Ala	Ser	Leu	Ser	Ser	Leu	Leu	980	985	990	
Asn	Arg	Leu	Asp	Pro	Val	Leu	Arg	Lys	Thr	Ala	Asn	Leu	Gly	Ser	Trp	995	1000	1005	
Gln	Ile	Ile	Ser	Pro	Val	Glu	Ala	Val	Gly	Tyr	Val	Val	Val	Val	Val	1010	1015	1020	
Asp	Glu	Leu	Leu	Ser	Val	Gln	Asn	Glu	Ile	Tyr	Glu	Lys	Pro	Thr	Thr	1025	1030	1035	
Ile	Leu	Val	Ala	Lys	Ser	Val	Lys	Gly	Glu	Glu	Glu	Ile	Pro	Asp	Asp	1040	1045	1050	
Gly	Ala	Val	Ala	Leu	Ile	Thr	Pro	Asp	Met	Pro	Asp	Val	Leu	Ser	Ser	1055	1060	1065	
His	Val	Ser	Val	Arg	Ala	Arg	Asn	Gly	Lys	Val	Cys	Phe	Ala	Thr	Thr	1070	1075	1080	
Cys	Phe	Asp	Pro	Asn	Ile	Leu	Ala	Asp	Leu	Gln	Ala	Lys	Glu	Gly	Gly	1085	1090	1095	
Arg	Ile	Leu	Leu	Leu	Lys	Pro	Thr	Pro	Ser	Asp	Ile	Ile	Tyr	Ser	Ser	1100	1105	1110	
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Gly Ala Lys Ser Arg Asn Ile Ala Tyr Leu Lys Gly Lys Val Pro
 1160 1165 1170

Ser Ser Val Gly Ile Pro Thr Ser Val Ala Leu Pro Phe Gly Val
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Phe Glu Lys Val Leu Ser Asp Asp Ile Asn Gln Gly Val Ala Lys
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Glu Leu Gln Ile Leu Met Lys Lys Leu Ser Glu Gly Asp Phe Ser
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Ala Leu Gly Glu Ile Arg Thr Thr Val Leu Asp Leu Ser Ala Pro
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Ala Gln Leu Val Lys Glu Leu Lys Glu Lys Met Gln Gly Ser Gly
 1235 1240 1245

Met Pro Trp Pro Gly Asp Glu Gly Pro Lys Arg Trp Glu Gln Ala
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Trp Met Ala Ile Lys Lys Val Trp Ala Ser Lys Trp Asn Glu Arg
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Ala Tyr Phe Ser Thr Arg Lys Val Lys Leu Asp His Asp Tyr Leu
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Cys Met Ala Val Leu Val Gln Glu Ile Ile Asn Ala Asp Tyr Ala
 1295 1300 1305

Phe Val Ile His Thr Thr Asn Pro Ser Ser Gly Asp Asp Ser Glu
 1310 1315 1320

Ile Tyr Ala Glu Val Val Arg Gly Leu Gly Glu Thr Leu Val Gly
 1325 1330 1335

Ala Tyr Pro Gly Arg Ala Leu Ser Phe Ile Cys Lys Lys Lys Asp
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Leu Asn Ser Pro Gln Val Leu Gly Tyr Pro Ser Lys Pro Ile Gly
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Leu Phe Ile Lys Arg Ser Ile Ile Phe Arg Ser Asp Ser Asn Gly
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Glu Asp Leu Glu Gly Tyr Ala Gly Ala Gly Leu Tyr Asp Ser Val
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Pro Met Asp Glu Glu Glu Lys Val Val Ile Asp Tyr Ser Ser Asp
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Pro Leu Ile Thr Asp Gly Asn Phe Arg Gln Thr Ile Leu Ser Asn
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Gln Thr Arg Pro Gln Met
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tctggcgga ccaaccgcg aaccgtacca tctcccgcc cgatcc atg tcg tcg	235
	Met Ser Ser
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Ala Val Ala Ser Ala Ala Ser Phe Leu Ala Leu Ala Ser Ala Ser Pro	
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Gly Ala Gly Arg Leu His Trp Pro Pro Trp Pro Pro Gln Arg Thr Ala	
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Arg Asp Gly Ala Val Ala Ala Leu Ala Ala Gly Lys Lys Asp Ala Gly	
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Ile Asp Asp Ala Ala Ala Ser Val Arg Gln Pro Arg Ala Leu Arg Gly	
70 75 80	
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Asp Arg Asp Ala Ala Glu Gly Gly Gly Pro Ser Pro Pro Ala Ala Arg	
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Gln Asp Ala Ala Arg Pro Pro Ser Met Asn Gly Met Pro Val Asn Gly	
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Pro Thr Pro Ala Arg Ala Pro His Pro Ser Thr Gln Asn Arg Ala Pro	
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Val Asn Gly Glu Asn Lys Ala Asn Val Ala Ser Pro Pro Thr Ser Ile	
165 170 175	
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Ala Glu Ala Ala Ala Ser Asp Ser Ala Ala Thr Ile Ser Ile Ser Asp	
180 185 190 195	
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Lys Ala Pro Glu Ser Val Val Pro Ala Glu Lys Thr Pro Pro Ser Ser	
200 205 210	
ggc tca aat ttc gag tcc tcg gcc tct gct ccc ggg tct gac act gtc	907
Gly Ser Asn Phe Glu Ser Ser Ala Ser Ala Pro Gly Ser Asp Thr Val	
215 220 225	
agc gac gtg gaa caa gaa ctg aag aag ggt gcg gtc gtt gtc gaa gaa	955
Ser Asp Val Glu Gln Glu Leu Lys Lys Gly Ala Val Val Val Glu Glu	
230 235 240	
gct cca aag cca aag gct ctt tcg ccg cct gca gcc ccc gct gta caa	1003
Ala Pro Lys Pro Lys Ala Leu Ser Pro Pro Ala Ala Pro Ala Val Gln	
245 250 255	
gaa gac ctt tgg gat ttc aag aaa tac att ggt ttc gag gag ccc gtg	1051
Glu Asp Leu Trp Asp Phe Lys Lys Tyr Ile Gly Phe Glu Glu Pro Val	
260 265 270 275	
gag gcc aag gat gat ggc cgg gct gtc gca gat gat gcg ggc tcc ttt	1099

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gaa	cac	cac	cag	aat	cac	gac	tcc	gga	cct	ttg	gca	ggg	gag	aat	gtc		1147
Glu	His	His	Gln	Asn	His	Asp	Ser	Gly	Pro	Leu	Ala	Gly	Glu	Asn	Val		
			295					300					305				
atg	aac	gtg	gtc	gtc	gtg	gct	gct	gag	tgt	tct	ccc	tgg	tgc	aaa	aca		1195
Met	Asn	Val	Val	Val	Val	Ala	Ala	Glu	Cys	Ser	Pro	Trp	Cys	Lys	Thr		
		310					315					320					
ggt	ggt	ctg	gga	gat	ggt	gcg	ggt	gct	ctg	ccc	aag	gct	ttg	gca	aag		1243
Gly	Gly	Leu	Gly	Asp	Val	Ala	Gly	Ala	Leu	Pro	Lys	Ala	Leu	Ala	Lys		
		325					330				335						
aga	gga	cat	cgt	ggt	atg	ggt	gtg	gta	cca	agg	tat	ggg	gac	tat	gaa		1291
Arg	Gly	His	Arg	Val	Met	Val	Val	Val	Pro	Arg	Tyr	Gly	Asp	Tyr	Glu		
					345					350					355		
gaa	gcc	tac	gat	gtc	gga	gtc	cga	aaa	tac	tac	aag	gct	gct	gga	cag		1339
Glu	Ala	Tyr	Asp	Val	Gly	Val	Arg	Lys	Tyr	Tyr	Lys	Ala	Ala	Gly	Gln		
				360					365					370			
gat	atg	gaa	gtg	aat	tat	ttc	cat	gct	tat	atc	gat	gga	ggt	gat	ttt		1387
Asp	Met	Glu	Val	Asn	Tyr	Phe	His	Ala	Tyr	Ile	Asp	Gly	Val	Asp	Phe		
				375				380						385			
gtg	ttc	att	gac	gct	cct	ctc	ttc	cga	cac	cgt	cag	gaa	gac	att	tat		1435
Val	Phe	Ile	Asp	Ala	Pro	Leu	Phe	Arg	His	Arg	Gln	Glu	Asp	Ile	Tyr		
			390					395				400					
ggg	ggc	agc	aga	cag	gaa	att	atg	aag	cgc	atg	att	ttg	ttc	tgc	aag		1483
Gly	Gly	Ser	Arg	Gln	Glu	Ile	Met	Lys	Arg	Met	Ile	Leu	Phe	Cys	Lys		
		405				410					415						
gcc	gct	ggt	gag	ggt	cca	tgg	cac	ggt	cca	tgc	ggc	ggt	gtc	cct	tat		1531
Ala	Ala	Val	Glu	Val	Pro	Trp	His	Val	Pro	Cys	Gly	Gly	Val	Pro	Tyr		
					425					430				435			
ggg	gat	gga	aat	ctg	gtg	ttt	att	gca	aat	gat	tgg	cac	acg	gca	ctc		1579
Gly	Asp	Gly	Asn	Leu	Val	Phe	Ile	Ala	Asn	Asp	Trp	His	Thr	Ala	Leu		
				440					445					450			
ctg	cct	gtc	tat	ctg	aaa	gca	tat	tac	agg	gac	cat	ggt	ttg	atg	cag		1627
Leu	Pro	Val	Tyr	Leu	Lys	Ala	Tyr	Tyr	Arg	Asp	His	Gly	Leu	Met	Gln		
				455				460						465			
tac	act	cgg	tcc	att	atg	gtg	ata	cat	aac	atc	gct	cac	cag	ggc	cgt		1675
Tyr	Thr	Arg	Ser	Ile	Met	Val	Ile	His	Asn	Ile	Ala	His	Gln	Gly	Arg		
				470			475					480					
ggc	cct	gta	gat	gaa	ttc	ccg	ttc	acc	gag	ttg	cct	gag	cac	tac	ctg		1723
Gly	Pro	Val	Asp	Glu	Phe	Pro	Phe	Thr	Glu	Leu	Pro	Glu	His	Tyr	Leu		
				485			490				495						
gaa	cac	ttc	aga	ctg	tac	gac	ccc	gtg	ggt	ggt	gaa	cac	gcc	aac	tac		1771
Glu	His	Phe	Arg	Leu	Tyr	Asp	Pro	Val	Gly	Gly	Glu	His	Ala	Asn	Tyr		
					505					510					515		
ttc	gcc	gcc	ggc	ctg	aag	atg	gcg	gac	cag	ggt	gtc	gtg	gtg	agc	ccc		1819
Phe	Ala	Ala	Gly	Leu	Lys	Met	Ala	Asp	Gln	Val	Val	Val	Val	Val	Ser	Pro	
				520					525					530			
ggg	tac	ctg	tgg	gag	ctg	aag	acg	gtg	gag	ggc	ggc	tgg	ggg	ctt	cac		1867
Gly	Tyr	Leu	Trp	Glu	Leu	Lys	Thr	Val	Glu	Gly	Gly	Trp	Gly	Leu	His		
				535				540						545			
gac	atc	ata	cgg	cag	aac	gac	tgg	aag	acc	cgc	ggc	atc	gtc	aac	ggc		1915
Asp	Ile	Ile	Arg	Gln	Asn	Asp	Trp	Lys	Thr	Arg	Gly	Ile	Val	Asn	Gly		
				550			555						560				
atc	gac	aac	atg	gag	tgg	aac	ccc	gag	gtg	gac	gcc	cac	ctc	aag	tcg		1963
Ile	Asp	Asn	Met	Glu	Trp	Asn	Pro	Glu	Val	Asp	Ala	His	Leu	Lys	Ser		
				565			570				575						
gac	ggc	tac	acc	aac	ttc	tcc	ctg	agg	acg	ctg	gac	tcc	ggc	aag	cgg		2011
Asp	Gly	Tyr	Thr	Asn	Phe	Ser	Leu	Arg	Thr	Leu	Asp	Ser	Gly	Lys	Arg		
					585					590					595		
cag	tgc	aag	gag	gcc	ctg	cag	cgc	gag	ctg	ggc	ctg	cag	gtc	cgc	gcc		2059

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Gln	Cys	Lys	Glu	Ala	Leu	Gln	Arg	Glu	Leu	Gly	Leu	Gln	Val	Arg	Ala	
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gac	gtg	ccg	ctg	ctc	ggc	ttc	atc	ggc	cgc	ctg	gac	ggg	cag	aag	ggc	2107
Asp	Val	Pro	Leu	Leu	Gly	Phe	Ile	Gly	Arg	Leu	Asp	Gly	Gln	Lys	Gly	
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gtg	gag	atc	atc	gcg	gac	gcc	atg	ccc	tgg	atc	gtg	agc	cag	gac	gtg	2155
Val	Glu	Ile	Ile	Ala	Asp	Ala	Met	Pro	Trp	Ile	Val	Ser	Gln	Asp	Val	
			630				635						640			
cag	ctg	gtg	atg	ctg	ggc	acc	ggg	cgc	cac	gac	ctg	gag	agc	atg	ctg	2203
Gln	Leu	Val	Met	Leu	Gly	Thr	Gly	Arg	His	Asp	Leu	Glu	Ser	Met	Leu	
			645			650					655					
cag	cac	ttc	gag	cgg	gag	cac	cac	gac	aag	gtg	cgc	ggg	tgg	gtg	ggg	2251
Gln	His	Phe	Glu	Arg	Glu	His	His	Asp	Lys	Val	Arg	Gly	Trp	Val	Gly	
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ttc	tcc	gtg	cgc	ctg	gcg	cac	cgg	atc	acg	gcg	ggg	gcg	gac	gcg	ctc	2299
Phe	Ser	Val	Arg	Leu	Ala	His	Arg	Ile	Thr	Ala	Gly	Ala	Asp	Ala	Leu	
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ctc	atg	ccc	tcc	cgg	ttc	gag	ccg	tgc	ggg	ctg	aac	cag	ctc	tac	gcc	2347
Leu	Met	Pro	Ser	Arg	Phe	Glu	Pro	Cys	Gly	Leu	Asn	Gln	Leu	Tyr	Ala	
			695					700					705			
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Met	Ala	Tyr	Gly	Thr	Val	Pro	Val	Val	His	Ala	Val	Gly	Gly	Leu	Arg	
			710				715					720				
gac	acc	gtg	ccg	ccg	ttc	gac	ccc	ttc	aac	cac	tcc	ggg	ctc	ggg	tgg	2443
Asp	Thr	Val	Pro	Pro	Phe	Asp	Pro	Phe	Asn	His	Ser	Gly	Leu	Gly	Trp	
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acg	ttc	gac	cgc	gcc	gag	gcg	cac	aag	ctg	atc	gag	gcg	ctc	ggg	cac	2491
Thr	Phe	Asp	Arg	Ala	Glu	Ala	His	Lys	Leu	Ile	Glu	Ala	Leu	Gly	His	
				740		745				750					755	
tgc	ctc	cgc	acc	tac	cga	gac	ttc	aag	gag	agc	tgg	agg	gcc	ctc	cag	2539
Cys	Leu	Arg	Thr	Tyr	Arg	Asp	Phe	Lys	Glu	Ser	Trp	Arg	Ala	Leu	Gln	
				760					765					770		
gag	cgc	ggc	atg	tcg	cag	gac	ttc	agc	tgg	gag	cac	gcc	gcc	aag	ctc	2587
Glu	Arg	Gly	Met	Ser	Gln	Asp	Phe	Ser	Trp	Glu	His	Ala	Ala	Lys	Leu	
			775					780						785		
tac	gag	gac	gtc	ctc	gtc	aag	gcc	aag	tac	cag	tgg	tgaacgctag				2633
Tyr	Glu	Asp	Val	Leu	Val	Lys	Ala	Lys	Tyr	Gln	Trp					
			790				795									
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Arg	Thr	Ala	Arg	Asp	Gly	Ala	Val	Ala	Ala	Leu	Ala	Ala	Gly	Lys	Lys
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Leu	Arg	Gly	Gly	Ala	Ala	Thr	Lys	Val	Ala	Glu	Arg	Arg	Asp	Pro	Val
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Lys	Thr	Leu	Asp	Arg	Asp	Ala	Ala	Glu	Gly	Gly	Gly	Pro	Ser	Pro	Pro
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Ala	Ala	Arg	Gln	Asp	Ala	Ala	Arg	Pro	Pro	Ser	Met	Asn	Gly	Met	Pro
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Val	Asn	Gly	Glu	Asn	Lys	Ser	Thr	Gly	Gly	Gly	Gly	Ala	Thr	Lys	Asp
	130				135								140		
Ser	Gly	Leu	Pro	Thr	Pro	Ala	Arg	Ala	Pro	His	Pro	Ser	Thr	Gln	Asn
	145				150					155					160
Arg	Ala	Pro	Val	Asn	Gly	Glu	Asn	Lys	Ala	Asn	Val	Ala	Ser	Pro	Pro
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Thr	Ser	Ile	Ala	Glu	Ala	Ala	Ala	Ser	Asp	Ser	Ala	Ala	Thr	Ile	Ser
				180				185					190		
Ile	Ser	Asp	Lys	Ala	Pro	Glu	Ser	Val	Val	Pro	Ala	Glu	Lys	Thr	Pro
		195						200					205		
Pro	Ser	Ser	Gly	Ser	Asn	Phe	Glu	Ser	Ser	Ala	Ser	Ala	Pro	Gly	Ser
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Asp	Thr	Val	Ser	Asp	Val	Glu	Gln	Glu	Leu	Lys	Lys	Gly	Ala	Val	Val
	225				230					235					240
Val	Glu	Glu	Ala	Pro	Lys	Pro	Lys	Ala	Leu	Ser	Pro	Pro	Ala	Ala	Pro
				245						250				255	
Ala	Val	Gln	Glu	Asp	Leu	Trp	Asp	Phe	Lys	Lys	Tyr	Ile	Gly	Phe	Glu
				260				265					270		
Glu	Pro	Val	Glu	Ala	Lys	Asp	Asp	Gly	Arg	Ala	Val	Ala	Asp	Asp	Ala
		275					280						285		
Gly	Ser	Phe	Glu	His	His	Gln	Asn	His	Asp	Ser	Gly	Pro	Leu	Ala	Gly
		290					295				300				
Glu	Asn	Val	Met	Asn	Val	Val	Val	Val	Ala	Ala	Glu	Cys	Ser	Pro	Trp
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Cys	Lys	Thr	Gly	Gly	Leu	Gly	Asp	Val	Ala	Gly	Ala	Leu	Pro	Lys	Ala
				325						330				335	
Leu	Ala	Lys	Arg	Gly	His	Arg	Val	Met	Val	Val	Val	Pro	Arg	Tyr	Gly
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Asp	Tyr	Glu	Glu	Ala	Tyr	Asp	Val	Gly	Val	Arg	Lys	Tyr	Tyr	Lys	Ala
		355					360						365		
Ala	Gly	Gln	Asp	Met	Glu	Val	Asn	Tyr	Phe	His	Ala	Tyr	Ile	Asp	Gly
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Val	Asp	Phe	Val	Phe	Ile	Asp	Ala	Pro	Leu	Phe	Arg	His	Arg	Gln	Glu
		385			390					395					400
Asp	Ile	Tyr	Gly	Gly	Ser	Arg	Gln	Glu	Ile	Met	Lys	Arg	Met	Ile	Leu
				405						410				415	
Phe	Cys	Lys	Ala	Ala	Val	Glu	Val	Pro	Trp	His	Val	Pro	Cys	Gly	Gly
				420				425					430		
Val	Pro	Tyr	Gly	Asp	Gly	Asn	Leu	Val	Phe	Ile	Ala	Asn	Asp	Trp	His
		435					440						445		
Thr	Ala	Leu	Leu	Pro	Val	Tyr	Leu	Lys	Ala	Tyr	Tyr	Arg	Asp	His	Gly
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Leu Met Gln Tyr Thr Arg Ser Ile Met Val Ile His Asn Ile Ala His
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 Gln Gly Arg Gly Pro Val Asp Glu Phe Pro Phe Thr Glu Leu Pro Glu
 485 490 495
 His Tyr Leu Glu His Phe Arg Leu Tyr Asp Pro Val Gly Gly Glu His
 500 505 510
 Ala Asn Tyr Phe Ala Ala Gly Leu Lys Met Ala Asp Gln Val Val Val
 515 520 525
 Val Ser Pro Gly Tyr Leu Trp Glu Leu Lys Thr Val Glu Gly Gly Trp
 530 535 540
 Gly Leu His Asp Ile Ile Arg Gln Asn Asp Trp Lys Thr Arg Gly Ile
 545 550 555 560
 Val Asn Gly Ile Asp Asn Met Glu Trp Asn Pro Glu Val Asp Ala His
 565 570 575
 Leu Lys Ser Asp Gly Tyr Thr Asn Phe Ser Leu Arg Thr Leu Asp Ser
 580 585 590
 Gly Lys Arg Gln Cys Lys Glu Ala Leu Gln Arg Glu Leu Gly Leu Gln
 595 600 605
 Val Arg Ala Asp Val Pro Leu Leu Gly Phe Ile Gly Arg Leu Asp Gly
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 Gln Lys Gly Val Glu Ile Ile Ala Asp Ala Met Pro Trp Ile Val Ser
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 Gln Asp Val Gln Leu Val Met Leu Gly Thr Gly Arg His Asp Leu Glu
 645 650 655
 Ser Met Leu Gln His Phe Glu Arg Glu His His Asp Lys Val Arg Gly
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 Trp Val Gly Phe Ser Val Arg Leu Ala His Arg Ile Thr Ala Gly Ala
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 Asp Ala Leu Leu Met Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln
 690 695 700
 Leu Tyr Ala Met Ala Tyr Gly Thr Val Pro Val Val His Ala Val Gly
 705 710 715 720
 Gly Leu Arg Asp Thr Val Pro Pro Phe Asp Pro Phe Asn His Ser Gly
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 Leu Gly Trp Thr Phe Asp Arg Ala Glu Ala His Lys Leu Ile Glu Ala
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gcg ggt gtt gtc agg cgg gct gat gac ggg gag aac gag gcc gca gtg Ala Gly Val Val Arg Arg Ala Asp Asp Gly Glu Asn Glu Ala Ala Val 65 70 75 80			240
gag cgg gcc ggc gag gac gat gac gag gag gag gag ttc tcg tcc ggg Glu Arg Ala Gly Glu Asp Asp Asp Glu Glu Glu Glu Phe Ser Ser Gly 85 90 95			288
gcc tgg cag cca ccg cgt tca agg cgc ggt gga gtt ggc aag gtc ctc Ala Trp Gln Pro Pro Arg Ser Arg Arg Gly Gly Val Gly Lys Val Leu 100 105 110			336
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ctc tgg gag ctg aag acg acg gag ggc ggc tgg ggc ctc cac gac atc Leu Trp Glu Leu Lys Thr Thr Glu Gly Gly Trp Gly Leu His Asp Ile 545 550 555 560			1680
ata cgg gag aac gac tgg aag atg aac ggc atc gtg aac ggc atc gac Ile Arg Glu Asn Asp Trp Lys Met Asn Gly Ile Val Asn Gly Ile Asp 565 570 575			1728
tac cgg gag tgg aac ccg gag gtg gac gtg cac ctg cag tcc gac ggc Tyr Arg Glu Trp Asn Pro Glu Val Asp Val His Leu Gln Ser Asp Gly 580 585 590			1776
tac gcc aac tac acc gtg gcc tcg ctg gac tcc agc aag ccg ccg tgc Tyr Ala Asn Tyr Thr Val Ala Ser Leu Asp Ser Ser Lys Pro Arg Cys 595 600 605			1824
aag gcg gcg ctg cag cgc gag ctg ggg ctg gag gtg cgc gac gac gtg Lys Ala Ala Leu Gln Arg Glu Leu Gly Leu Glu Val Arg Asp Asp Val 610 615 620			1872
ccg ctg atc ggg ttc atc ggg ccg ctc gac ggg cag aaa ggt gtg gac Pro Leu Ile Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Asp 625 630 635 640			1920
atc atc ggc gac gcg atg ccg tgg atc gcc ggg cag gac gtg cag ctg Ile Ile Gly Asp Ala Met Pro Trp Ile Ala Gly Gln Asp Val Gln Leu 645 650 655			1968
gtg ctg ctg ggc tcc ggc cgc cgc gac ctg gag gtg atg ctg cag ccg Val Leu Leu Gly Ser Gly Arg Arg Asp Leu Glu Val Met Leu Gln Arg 2016			

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660	665	670	
ttc gag gcg cag cac aac agc aag gtg cgc ggg tgg gtg ggg ttc tcg Phe Glu Ala Gln His Asn Ser Lys Val Arg Gly Trp Val Gly Phe Ser 675 680 685			2064
gtg aag atg gcg cac cgg atc acg gcg ggc gcc gac gtg ctg gtc atg Val Lys Met Ala His Arg Ile Thr Ala Gly Ala Asp Val Leu Val Met 690 695 700			2112
ccg tcg cgg ttc gag ccg tgc ggc ctc aac cag ctc tac gcc atg gcg Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala 705 710 715 720			2160
tac ggc acc gtc ccc gtc gtg cac gcc gtc ggc ggg ctg agg gac acc Tyr Gly Thr Val Val Val His Ala Val Gly Gly Leu Arg Asp Thr 725 730 735			2208
gtg tcg gcg ttc gac ccg ttc gag gac acc ggc ctc ggg tgg acg ttc Val Ser Ala Phe Asp Pro Phe Glu Asp Thr Gly Leu Gly Trp Thr Phe 740 745 750			2256
gac cgc gcc gag ccg cac aag ctc atc gag gcg ctc ggc cac tgc ctg Asp Arg Ala Glu Pro His Lys Leu Ile Glu Ala Leu Gly His Cys Leu 755 760 765			2304
gag acg tac cgc aag tac aag gag agc tgg agg ggg ctc cag gtg cgc Glu Thr Tyr Arg Lys Tyr Lys Glu Ser Trp Arg Gly Leu Gln Val Arg 770 775 780			2352
ggc atg tcg cag gac ctc agc tgg gac cac gcc gcc gag ctc tac gag Gly Met Ser Gln Asp Leu Ser Trp Asp His Ala Ala Glu Leu Tyr Glu 785 790 795 800			2400
gag gtc ctt gtc aag gcc aag tac caa tgg tga Glu Val Leu Val Lys Ala Lys Tyr Gln Trp 805 810			2433

<210> SEQ ID NO 6
 <211> LENGTH: 810
 <212> TYPE: PRT
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 6

Met Ser Ser Ala Val Val Ala Ser Ser Thr Thr Phe Leu Val Ala Leu 1 5 10 15
Ala Ser Ser Ala Ser Arg Gly Gly Pro Arg Arg Gly Arg Val Val Gly 20 25 30
Val Ala Ala Pro Pro Ala Leu Leu Tyr Asp Gly Arg Ala Gly Arg Leu 35 40 45
Ala Leu Arg Ala Pro Pro Pro Pro Arg Pro Arg Pro Arg Arg Arg Asp 50 55 60
Ala Gly Val Val Arg Arg Ala Asp Asp Gly Glu Asn Glu Ala Ala Val 65 70 75 80
Glu Arg Ala Gly Glu Asp Asp Asp Glu Glu Glu Glu Phe Ser Ser Gly 85 90 95
Ala Trp Gln Pro Pro Arg Ser Arg Arg Gly Gly Val Gly Lys Val Leu 100 105 110
Lys Arg Arg Gly Thr Val Pro Pro Val Gly Arg Tyr Gly Ser Gly Gly 115 120 125
Asp Ala Ala Arg Val Arg Gly Ala Ala Ala Pro Ala Pro Ala Pro Thr 130 135 140
Gln Asp Ala Ala Ser Ser Lys Asn Gly Ala Leu Leu Ser Gly Arg Asp 145 150 155 160
Asp Asp Thr Pro Ala Ser Arg Asn Gly Ser Val Val Thr Gly Ala Asp 165 170 175
Lys Pro Ala Ala Ala Thr Pro Pro Val Thr Ile Thr Lys Leu Pro Ala

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180					185					190					
Pro	Asp	Ser	Pro	Val	Ile	Leu	Pro	Ser	Val	Asp	Lys	Pro	Gln	Pro	Glu
		195					200					205			
Phe	Val	Ile	Pro	Asp	Ala	Thr	Ala	Pro	Ala	Pro	Pro	Pro	Pro	Gly	Ser
	210					215					220				
Asn	Pro	Arg	Ser	Ser	Ala	Pro	Leu	Pro	Lys	Pro	Asp	Asn	Ser	Glu	Phe
						230					235				240
Ala	Glu	Asp	Lys	Ser	Ala	Lys	Val	Val	Glu	Ser	Ala	Pro	Lys	Pro	Lys
						245					250				255
Ala	Thr	Arg	Ser	Ser	Pro	Ile	Pro	Ala	Val	Glu	Glu	Glu	Thr	Trp	Asp
						260					265				270
Phe	Lys	Lys	Tyr	Phe	Asp	Leu	Asn	Glu	Pro	Asp	Ala	Ala	Glu	Asp	Gly
							280					285			
Asp	Asp	Asp	Asp	Asp	Trp	Ala	Asp	Ser	Asp	Ala	Ser	Asp	Ser	Glu	Ile
						295					300				
Asp	Gln	Asp	Asp	Asp	Ser	Gly	Pro	Leu	Ala	Gly	Glu	Asn	Val	Met	Asn
						310					315				320
Val	Ile	Val	Val	Ala	Ala	Glu	Cys	Ser	Pro	Trp	Cys	Lys	Thr	Gly	Gly
						325					330				335
Leu	Gly	Asp	Val	Ala	Gly	Ala	Leu	Pro	Lys	Ala	Leu	Ala	Arg	Arg	Gly
						340					345				350
His	Arg	Val	Met	Val	Val	Val	Pro	Arg	Tyr	Gly	Asp	Tyr	Ala	Glu	Ala
							360					365			
Gln	Asp	Val	Gly	Ile	Arg	Lys	Tyr	Tyr	Lys	Ala	Ala	Gly	Gln	Asp	Leu
						375					380				
Glu	Val	Lys	Tyr	Phe	His	Ala	Phe	Ile	Asp	Gly	Val	Asp	Phe	Val	Phe
						390					395				400
Ile	Asp	Ala	Pro	Leu	Phe	Arg	His	Arg	Gln	Asp	Asp	Ile	Tyr	Gly	Gly
						405					410				415
Asn	Arg	Gln	Glu	Ile	Met	Lys	Arg	Met	Ile	Leu	Phe	Cys	Lys	Ala	Ala
						420					425				430
Val	Glu	Val	Pro	Trp	His	Val	Pro	Cys	Gly	Gly	Val	Pro	Tyr	Gly	Asp
						435					440				445
Gly	Asn	Leu	Val	Phe	Leu	Ala	Asn	Asp	Trp	His	Thr	Ala	Leu	Leu	Pro
						455					460				
Val	Tyr	Leu	Lys	Ala	Tyr	Tyr	Arg	Asp	Asn	Gly	Met	Met	Gln	Tyr	Thr
						470					475				480
Arg	Ser	Val	Leu	Val	Ile	His	Asn	Ile	Ala	Tyr	Gln	Gly	Arg	Gly	Pro
						485					490				495
Val	Asp	Glu	Phe	Pro	Tyr	Met	Glu	Leu	Pro	Glu	His	Tyr	Leu	Asp	His
						500					505				510
Phe	Lys	Leu	Tyr	Asp	Pro	Val	Gly	Gly	Glu	His	Ala	Asn	Ile	Phe	Gly
						515					520				525
Ala	Gly	Leu	Lys	Met	Ala	Asp	Arg	Val	Val	Thr	Val	Ser	Pro	Gly	Tyr
						535					540				
Leu	Trp	Glu	Leu	Lys	Thr	Thr	Glu	Gly	Gly	Trp	Gly	Leu	His	Asp	Ile
						550					555				560
Ile	Arg	Glu	Asn	Asp	Trp	Lys	Met	Asn	Gly	Ile	Val	Asn	Gly	Ile	Asp
						565					570				575
Tyr	Arg	Glu	Trp	Asn	Pro	Glu	Val	Asp	Val	His	Leu	Gln	Ser	Asp	Gly
						580					585				590
Tyr	Ala	Asn	Tyr	Thr	Val	Ala	Ser	Leu	Asp	Ser	Ser	Lys	Pro	Arg	Cys
						595					600				605

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Lys Ala Ala Leu Gln Arg Glu Leu Gly Leu Glu Val Arg Asp Asp Val
 610 615 620
 Pro Leu Ile Gly Phe Ile Gly Arg Leu Asp Gly Gln Lys Gly Val Asp
 625 630 635 640
 Ile Ile Gly Asp Ala Met Pro Trp Ile Ala Gly Gln Asp Val Gln Leu
 645 650 655
 Val Leu Leu Gly Ser Gly Arg Arg Asp Leu Glu Val Met Leu Gln Arg
 660 665 670
 Phe Glu Ala Gln His Asn Ser Lys Val Arg Gly Trp Val Gly Phe Ser
 675 680 685
 Val Lys Met Ala His Arg Ile Thr Ala Gly Ala Asp Val Leu Val Met
 690 695 700
 Pro Ser Arg Phe Glu Pro Cys Gly Leu Asn Gln Leu Tyr Ala Met Ala
 705 710 715 720
 Tyr Gly Thr Val Pro Val Val His Ala Val Gly Gly Leu Arg Asp Thr
 725 730 735
 Val Ser Ala Phe Asp Pro Phe Glu Asp Thr Gly Leu Gly Trp Thr Phe
 740 745 750
 Asp Arg Ala Glu Pro His Lys Leu Ile Glu Ala Leu Gly His Cys Leu
 755 760 765
 Glu Thr Tyr Arg Lys Tyr Lys Glu Ser Trp Arg Gly Leu Gln Val Arg
 770 775 780
 Gly Met Ser Gln Asp Leu Ser Trp Asp His Ala Ala Glu Leu Tyr Glu
 785 790 795 800
 Glu Val Leu Val Lys Ala Lys Tyr Gln Trp
 805 810

<210> SEQ ID NO 7
 <211> LENGTH: 1830
 <212> TYPE: DNA
 <213> ORGANISM: Oryza sativa
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1827)

<400> SEQUENCE: 7

atg tcg gct ctc acc acg tcc cag ctc gcc acc tcg gcc acc ggc ttc 48
 Met Ser Ala Leu Thr Ser Gln Leu Ala Thr Ser Ala Thr Gly Phe
 1 5 10 15
 ggc atc gcc gac agg tcg gcg ccg tcg tcg ctg ctc cgc cac ggg ttc 96
 Gly Ile Ala Asp Arg Ser Ala Pro Ser Ser Leu Leu Arg His Gly Phe
 20 25 30
 cag ggc ctc aag ccc cgc agc ccc gcc ggc ggc gac gcg acg tcg ctc 144
 Gln Gly Leu Lys Pro Arg Ser Pro Ala Gly Gly Asp Ala Thr Ser Leu
 35 40 45
 agc gtg acg acc agc gcg cgc gcg acg ccc aag cag cag cgg tcg gtg 192
 Ser Val Thr Thr Ser Ala Arg Ala Thr Pro Lys Gln Gln Arg Ser Val
 50 55 60
 cag cgt ggc agc cgg agg ttc ccc tcc gtc gtc gtg tac gcc acc ggc 240
 Gln Arg Gly Ser Arg Arg Phe Pro Ser Val Val Val Tyr Ala Thr Gly
 65 70 75 80
 gcc ggc atg aac gtc gtg ttc gtc gcc gcc gag atg gcc ccc tgg agc 288
 Ala Gly Met Asn Val Val Phe Val Gly Ala Glu Met Ala Pro Trp Ser
 85 90 95
 aag acc ggc ggc ctc ggt gac gtc ctc ggt gcc ctc ccc cct gcc atg 336
 Lys Thr Gly Gly Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Met
 100 105 110
 gct gcg aat ggc cac agg gtc atg gtg atc tct cct cgg tac gac cag 384
 Ala Ala Asn Gly His Arg Val Met Val Ile Ser Pro Arg Tyr Asp Gln

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115	120	125	
tac aag gac gct tgg gat acc agc gtt gtg gct gag atc aag gtt gca Tyr Lys Asp Ala Trp Asp Thr Ser Val Val Ala Glu Ile Lys Val Ala 130 135 140			432
gac agg tac gag agg gtg agg ttt ttc cat tgc tac aag cgt gga gtc Asp Arg Tyr Glu Arg Val Arg Phe Phe His Cys Tyr Lys Arg Gly Val 145 150 155 160			480
gac cgt gtg ttc atc gac cat ccg tca ttc ctg gag aag gtt tgg gga Asp Arg Val Phe Ile Asp His Pro Ser Phe Leu Glu Lys Val Trp Gly 165 170 175			528
aag acc ggt gag aag atc tac gga cct gac act gga gtt gat tac aaa Lys Thr Gly Glu Lys Ile Tyr Gly Pro Asp Thr Gly Val Asp Tyr Lys 180 185 190			576
gac aac cag atg cgt ttc agc ctt ctt tgc cag gca gca ctc gag gct Asp Asn Gln Met Arg Phe Ser Leu Leu Cys Gln Ala Ala Leu Glu Ala 195 200 205			624
cct agg atc cta aac ctc aac aac aac cca tac ttc aaa gga act tat Pro Arg Ile Leu Asn Leu Asn Asn Asn Pro Tyr Phe Lys Gly Thr Tyr 210 215 220			672
ggt gag gat gtt gtg ttc gtc tgc aac gac tgg cac act ggc cca ctg Gly Glu Asp Val Val Phe Val Cys Asn Asp Trp His Thr Gly Pro Leu 225 230 235 240			720
gcg agc tac ctg aag aac aac tac cag ccc aat ggc atc tac agg aat Ala Ser Tyr Leu Lys Asn Asn Tyr Gln Pro Asn Gly Ile Tyr Arg Asn 245 250 255			768
gca aag gtt gct ttc tgc atc cac aac atc tcc tac cag ggc cgt ttc Ala Lys Val Ala Phe Cys Ile His Asn Ile Ser Tyr Gln Gly Arg Phe 260 265 270			816
gct ttc gag gat tac cct gag ctg aac ctc tcc gag agg ttc agg tca Ala Phe Glu Asp Tyr Pro Glu Leu Asn Leu Ser Glu Arg Phe Arg Ser 275 280 285			864
tcc ttc gat ttc atc gac ggg tat gac acg ccg gtg gag ggc agg aag Ser Phe Asp Phe Ile Asp Gly Tyr Asp Thr Pro Val Glu Gly Arg Lys 290 295 300			912
atc aac tgg atg aag gcc gga atc ctg gaa gcc gac agg gtg ctc acc Ile Asn Trp Met Lys Ala Gly Ile Leu Glu Ala Asp Arg Val Leu Thr 305 310 315 320			960
gtg agc ccg tac tac gcc gag gag ctc atc tcc ggc atc gcc agg gga Val Ser Pro Tyr Tyr Ala Glu Glu Leu Ile Ser Gly Ile Ala Arg Gly 325 330 335			1008
tgc gag ctc gac aac atc atg cgg ctc acc ggc atc acc ggc atc gtc Cys Glu Leu Asp Asn Ile Met Arg Leu Thr Gly Ile Thr Gly Ile Val 340 345 350			1056
aac ggc atg gac gtc agc gag tgg gat ccc agc aag gac aag tac atc Asn Gly Met Asp Val Ser Glu Trp Asp Pro Ser Lys Asp Lys Tyr Ile 355 360 365			1104
acc gcc aag tac gac gca acc acg gca atc gag gcg aag gcg ctg aac Thr Ala Lys Tyr Asp Ala Thr Thr Ala Ile Glu Ala Lys Ala Leu Asn 370 375 380			1152
aag gag gcg ttg cag gcg gag gcg ggt ctt ccg gtc gac agg aaa atc Lys Glu Ala Leu Gln Ala Glu Ala Gly Leu Pro Val Asp Arg Lys Ile 385 390 395 400			1200
cca ctg atc gcg ttc atc ggc agg ctg gag gaa cag aag ggc tct gac Pro Leu Ile Ala Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly Ser Asp 405 410 415			1248
gtc atg gcc gcc gcc atc ccg gag ctc atg cag gag gac gtc cag atc Val Met Ala Ala Ala Ile Pro Glu Leu Met Gln Glu Asp Val Gln Ile 420 425 430			1296
gtt ctt ctg ggt act gga aag aag aag ttc gag aag ctg ctc aag agc Val Leu Leu Gly Thr Gly Lys Lys Lys Phe Glu Lys Leu Leu Lys Ser			1344

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435	440	445	
atg gag gag aag tat ccg ggc aag gtg agg gcc gtg gtg aag ttc aac			1392
Met Glu Glu Lys Tyr Pro Gly Lys Val Arg Ala Val Val Lys Phe Asn			
450	455	460	
gcg ccg ctt gct cat ctc atc atg gcc gga gcc gac gtg ctc gcc gtc			1440
Ala Pro Leu Ala His Leu Ile Met Ala Gly Ala Asp Val Leu Ala Val			
465	470	475	480
ccc agc cgc ttc gag ccc tgt gga ctc atc cag ctg cag ggg atg aga			1488
Pro Ser Arg Phe Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg			
	485	490	495
tac gga acg ccc tgt gct tgc gcg tcc acc ggt ggg ctc gtg gac acg			1536
Tyr Gly Thr Pro Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr			
	500	505	510
gtc atc gaa ggc aag act ggt ttc cac atg ggc cgt ctc agc gtc gac			1584
Val Ile Glu Gly Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp			
	515	520	525
tgc aag gtg gtg gag cca agc gac gtg aag aag gtg gcg gcc acc ctg			1632
Cys Lys Val Val Glu Pro Ser Asp Val Lys Lys Val Ala Ala Thr Leu			
	530	535	540
aag cgc gcc atc aag gtc gtc ggc acg ccg gcg tac gag gag atg gtc			1680
Lys Arg Ala Ile Lys Val Val Gly Thr Pro Ala Tyr Glu Glu Met Val			
545	550	555	560
agg aac tgc atg aac cag gac ctc tcc tgg aag ggg cct gcg aag aac			1728
Arg Asn Cys Met Asn Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn			
	565	570	575
tgg gag aat gtg ctc ctg ggc ctg ggc gtc gcc ggc agc gcg ccg ggg			1776
Trp Glu Asn Val Leu Leu Gly Leu Gly Val Ala Gly Ser Ala Pro Gly			
	580	585	590
atc gaa ggc gac gag atc gcg ccg ctc gcc aag gag aac gtg gct gct			1824
Ile Glu Gly Asp Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Ala			
	595	600	605
cct tga			1830
Pro			

<210> SEQ ID NO 8
 <211> LENGTH: 609
 <212> TYPE: PRT
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 8

Met Ser Ala Leu Thr Thr Ser Gln Leu Ala Thr Ser Ala Thr Gly Phe			
1	5	10	15
Gly Ile Ala Asp Arg Ser Ala Pro Ser Ser Leu Leu Arg His Gly Phe			
	20	25	30
Gln Gly Leu Lys Pro Arg Ser Pro Ala Gly Gly Asp Ala Thr Ser Leu			
	35	40	45
Ser Val Thr Thr Ser Ala Arg Ala Thr Pro Lys Gln Gln Arg Ser Val			
	50	55	60
Gln Arg Gly Ser Arg Arg Phe Pro Ser Val Val Val Tyr Ala Thr Gly			
65	70	75	80
Ala Gly Met Asn Val Val Phe Val Gly Ala Glu Met Ala Pro Trp Ser			
	85	90	95
Lys Thr Gly Gly Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Met			
	100	105	110
Ala Ala Asn Gly His Arg Val Met Val Ile Ser Pro Arg Tyr Asp Gln			
	115	120	125
Tyr Lys Asp Ala Trp Asp Thr Ser Val Val Ala Glu Ile Lys Val Ala			
	130	135	140

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Asp	Arg	Tyr	Glu	Arg	Val	Arg	Phe	Phe	His	Cys	Tyr	Lys	Arg	Gly	Val	145	150	155	160
Asp	Arg	Val	Phe	Ile	Asp	His	Pro	Ser	Phe	Leu	Glu	Lys	Val	Trp	Gly	165	170	175	
Lys	Thr	Gly	Glu	Lys	Ile	Tyr	Gly	Pro	Asp	Thr	Gly	Val	Asp	Tyr	Lys	180	185	190	
Asp	Asn	Gln	Met	Arg	Phe	Ser	Leu	Leu	Cys	Gln	Ala	Ala	Leu	Glu	Ala	195	200	205	
Pro	Arg	Ile	Leu	Asn	Leu	Asn	Asn	Asn	Pro	Tyr	Phe	Lys	Gly	Thr	Tyr	210	215	220	
Gly	Glu	Asp	Val	Val	Phe	Val	Cys	Asn	Asp	Trp	His	Thr	Gly	Pro	Leu	225	230	235	240
Ala	Ser	Tyr	Leu	Lys	Asn	Asn	Tyr	Gln	Pro	Asn	Gly	Ile	Tyr	Arg	Asn	245	250	255	
Ala	Lys	Val	Ala	Phe	Cys	Ile	His	Asn	Ile	Ser	Tyr	Gln	Gly	Arg	Phe	260	265	270	
Ala	Phe	Glu	Asp	Tyr	Pro	Glu	Leu	Asn	Leu	Ser	Glu	Arg	Phe	Arg	Ser	275	280	285	
Ser	Phe	Asp	Phe	Ile	Asp	Gly	Tyr	Asp	Thr	Pro	Val	Glu	Gly	Arg	Lys	290	295	300	
Ile	Asn	Trp	Met	Lys	Ala	Gly	Ile	Leu	Glu	Ala	Asp	Arg	Val	Leu	Thr	305	310	315	320
Val	Ser	Pro	Tyr	Tyr	Ala	Glu	Glu	Leu	Ile	Ser	Gly	Ile	Ala	Arg	Gly	325	330	335	
Cys	Glu	Leu	Asp	Asn	Ile	Met	Arg	Leu	Thr	Gly	Ile	Thr	Gly	Ile	Val	340	345	350	
Asn	Gly	Met	Asp	Val	Ser	Glu	Trp	Asp	Pro	Ser	Lys	Asp	Lys	Tyr	Ile	355	360	365	
Thr	Ala	Lys	Tyr	Asp	Ala	Thr	Thr	Ala	Ile	Glu	Ala	Lys	Ala	Leu	Asn	370	375	380	
Lys	Glu	Ala	Leu	Gln	Ala	Glu	Ala	Gly	Leu	Pro	Val	Asp	Arg	Lys	Ile	385	390	395	400
Pro	Leu	Ile	Ala	Phe	Ile	Gly	Arg	Leu	Glu	Glu	Gln	Lys	Gly	Ser	Asp	405	410	415	
Val	Met	Ala	Ala	Ala	Ile	Pro	Glu	Leu	Met	Gln	Glu	Asp	Val	Gln	Ile	420	425	430	
Val	Leu	Leu	Gly	Thr	Gly	Lys	Lys	Lys	Phe	Glu	Lys	Leu	Leu	Lys	Ser	435	440	445	
Met	Glu	Glu	Lys	Tyr	Pro	Gly	Lys	Val	Arg	Ala	Val	Val	Lys	Phe	Asn	450	455	460	
Ala	Pro	Leu	Ala	His	Leu	Ile	Met	Ala	Gly	Ala	Asp	Val	Leu	Ala	Val	465	470	475	480
Pro	Ser	Arg	Phe	Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	485	490	495	
Tyr	Gly	Thr	Pro	Cys	Ala	Cys	Ala	Ser	Thr	Gly	Gly	Leu	Val	Asp	Thr	500	505	510	
Val	Ile	Glu	Gly	Lys	Thr	Gly	Phe	His	Met	Gly	Arg	Leu	Ser	Val	Asp	515	520	525	
Cys	Lys	Val	Val	Glu	Pro	Ser	Asp	Val	Lys	Lys	Val	Ala	Ala	Thr	Leu	530	535	540	
Lys	Arg	Ala	Ile	Lys	Val	Val	Gly	Thr	Pro	Ala	Tyr	Glu	Glu	Met	Val	545	550	555	560
Arg	Asn	Cys	Met	Asn	Gln	Asp	Leu	Ser	Trp	Lys	Gly	Pro	Ala	Lys	Asn	565	570	575	

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Trp Glu Asn Val Leu Leu Gly Leu Gly Val Ala Gly Ser Ala Pro Gly
 580 585 590

Ile Glu Gly Asp Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Ala
 595 600 605

Pro

<210> SEQ ID NO 9
 <211> LENGTH: 1818
 <212> TYPE: DNA
 <213> ORGANISM: Triticum aestivum
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1815)

<400> SEQUENCE: 9

atg gcg gct ctg gtc acg tcg cag ctc gcc acc tcc ggc acc gtc ctc	48
Met Ala Ala Leu Val Thr Ser Gln Leu Ala Thr Ser Gly Thr Val Leu	
1 5 10 15	
ggc atc acc gac agg ttc cgg cgt gca ggt ttt cag ggt gtg agg ccc	96
Gly Ile Thr Asp Arg Phe Arg Arg Ala Gly Phe Gln Gly Val Arg Pro	
20 25 30	
cgg agc ccg gca gat gcg ccg ctc ggc atg agg act acc gga gcg agc	144
Arg Ser Pro Ala Asp Ala Pro Leu Gly Met Arg Thr Thr Gly Ala Ser	
35 40 45	
gcc gcc ccg aag caa caa agc cgg aaa gcg cac cgc ggg acc cgg cgg	192
Ala Ala Pro Lys Gln Gln Ser Arg Lys Ala His Arg Gly Thr Arg Arg	
50 55 60	
tgc ctc tcc atg gtg gtg cgc gcc acg ggc agc gcc ggc atg aac ctc	240
Cys Leu Ser Met Val Val Arg Ala Thr Gly Ser Ala Gly Met Asn Leu	
65 70 75 80	
gtg ttc gtc ggc gcc gag atg gcg ccc tgg agc aag acc ggc ggc ctc	288
Val Phe Val Gly Ala Glu Met Ala Pro Trp Ser Lys Thr Gly Gly Leu	
85 90 95	
ggc gac gtc ctc ggg ggc ctc ccc cca gcc atg gcc gcc aac ggt cac	336
Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Met Ala Ala Asn Gly His	
100 105 110	
cgg gtc atg gtc atc tcc ccg cgc tac gac cag tac aag gac gcc tgg	384
Arg Val Met Val Ile Ser Pro Arg Tyr Asp Gln Tyr Lys Asp Ala Trp	
115 120 125	
gac acc agc gtc gtc tcc gag atc aag gtc gcg gac gag tac gag agg	432
Asp Thr Ser Val Val Ser Glu Ile Lys Val Ala Asp Glu Tyr Glu Arg	
130 135 140	
gtg agg tac ttc cac tgc tac aag cgc ggg gtg gac cgc gtg ttc gtc	480
Val Arg Tyr Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe Val	
145 150 155 160	
gac cac ccg tgc ttc ctg gag aag gtc cgg ggc aag acc aag gag aag	528
Asp His Pro Cys Phe Leu Glu Lys Val Arg Gly Lys Thr Lys Glu Lys	
165 170 175	
atc tac ggg ccc gat gcc ggc acg gac tac gag gac aac cag cta cgc	576
Ile Tyr Gly Pro Asp Ala Gly Thr Asp Tyr Glu Asp Asn Gln Leu Arg	
180 185 190	
ttc agc ctg ctc tgc cag gca gcg ctt gag gca ccc agg atc ctc gac	624
Phe Ser Leu Leu Cys Gln Ala Ala Leu Glu Ala Pro Arg Ile Leu Asp	
195 200 205	
ctc aac aac aac cca tac ttc tcc gga ccc tac ggg gaa gac gtg gtg	672
Leu Asn Asn Asn Pro Tyr Phe Ser Gly Pro Tyr Gly Glu Asp Val Val	
210 215 220	
ttc gtg tgc aac gac tgg cac acg ggc ctt ctg gcc tgc tac ctc aag	720
Phe Val Cys Asn Asp Trp His Thr Gly Leu Leu Ala Cys Tyr Leu Lys	
225 230 235 240	

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agc aac tac cag tcc agt ggc atc tat agg acg gcc aag gta gcg ttc Ser Asn Tyr Gln Ser Ser Gly Ile Tyr Arg Thr Ala Lys Val Ala Phe 245 250 255	768
tgc atc cac aac atc tcg tat cag ggc cgc ttc tcc ttc gac gac ttc Cys Ile His Asn Ile Ser Tyr Gln Gly Arg Phe Ser Phe Asp Asp Phe 260 265 270	816
gcg cag ctc aac ctg ccc gac agg ttc aag tcg tcc ttc gac ttc atc Ala Gln Leu Asn Leu Pro Asp Arg Phe Lys Ser Ser Phe Asp Phe Ile 275 280 285	864
gac ggc tac gac aag ccg gtg gag ggg cgc aag atc aac tgg atg aag Asp Gly Tyr Asp Lys Pro Val Glu Gly Arg Lys Ile Asn Trp Met Lys 290 295 300	912
gcc ggg atc ctg cag gcc gac aag gtg ctc acg gtg agc ccc tac tac Ala Gly Ile Leu Gln Ala Asp Lys Val Leu Thr Val Ser Pro Tyr Tyr 305 310 315 320	960
gcg gag gag ctc atc tcc ggc gaa gcc agg ggc tgc gag ctc gac aac Ala Glu Glu Leu Ile Ser Gly Glu Ala Arg Gly Cys Glu Leu Asp Asn 325 330 335	1008
atc atg cgc ctc acg ggc atc acc ggc atc gtc aac ggc atg gac gtc Ile Met Arg Leu Thr Gly Ile Thr Gly Ile Val Asn Gly Met Asp Val 340 345 350	1056
agc gag tgg gac ccc gcc aag gac aag ttc ctc gcc gcc aac tac gac Ser Glu Trp Asp Pro Ala Lys Asp Lys Phe Leu Ala Ala Asn Tyr Asp 355 360 365	1104
gtc acc acc gcg ttg gag ggg aag gcg ctg aac aag gag gcg ctg cag Val Thr Thr Ala Leu Glu Gly Lys Ala Leu Asn Lys Glu Ala Leu Gln 370 375 380	1152
gcc gag gtg ggg ctg ccg gtg gac cgg aag gtg ccc ctg gtg gcc ttc Ala Glu Val Gly Leu Pro Val Asp Arg Lys Val Pro Leu Val Ala Phe 385 390 395 400	1200
atc ggc agg ctg gag gag cag aag ggc ccc gac gtg atg atc gcc gcc Ile Gly Arg Leu Glu Glu Gln Lys Gly Pro Asp Val Met Ile Ala Ala 405 410 415	1248
atc ccg gag atc ttg aag gag gag gac gtc cag atc gtt ctc ctg ggc Ile Pro Glu Ile Leu Lys Glu Glu Asp Val Gln Ile Val Leu Leu Gly 420 425 430	1296
acc ggg aag aag aag ttt gag cgg ctg ctc aag agc gtg gag gag aag Thr Gly Lys Lys Lys Phe Glu Arg Leu Leu Lys Ser Val Glu Glu Lys 435 440 445	1344
ttc ccg agc aag gtg agg gcc gtg gtc agg ttc aac gcg ccg ctg gct Phe Pro Ser Lys Val Arg Ala Val Val Arg Phe Asn Ala Pro Leu Ala 450 455 460	1392
cac cag atg atg gcc ggc gcc gac gtg ctc gcc gtc acc agc cgc ttc His Gln Met Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg Phe 465 470 475 480	1440
gag ccc tgc ggc ctc atc cag ctc cag ggg atg cgc tac gga acg ccg Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr Pro 485 490 495	1488
tgc gcg tgc gcg tcc acc ggc ggg ctc gtc gac acg atc atg gag ggc Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr Ile Met Glu Gly 500 505 510	1536
aag acc ggg ttc cac atg ggc cgc ctc agc gtc gac tgc aac gtg gtg Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp Cys Asn Val Val 515 520 525	1584
gag ccg gcc gac gtg aag aag gtg gtg acc acc ctg aag cgc gcc gtc Glu Pro Ala Asp Val Lys Lys Val Val Thr Thr Leu Lys Arg Ala Val 530 535 540	1632
aag gtc gtc ggc acg cca gcc tac cat gag atg gtc aag aac tgc atg Lys Val Val Gly Thr Pro Ala Tyr His Glu Met Val Lys Asn Cys Met 545 550 555 560	1680

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atc cag gat ctc tcc tgg aag ggg cca gcc aag aac tgg gag gac gtg	1728
Ile Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn Trp Glu Asp Val	
565 570 575	
ctt ctg gaa ctg ggg gtc gag ggg agc gag cca ggg gtc atc ggc gag	1776
Leu Leu Glu Leu Gly Val Glu Gly Ser Glu Pro Gly Val Ile Gly Glu	
580 585 590	
gag att gcg ccg ctc gcc atg gag aac gtc gcc gct ccc tga	1818
Glu Ile Ala Pro Leu Ala Met Glu Asn Val Ala Ala Pro	
595 600 605	

<210> SEQ ID NO 10

<211> LENGTH: 605

<212> TYPE: PRT

<213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 10

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1 5 10 15	
Gly Ile Thr Asp Arg Phe Arg Arg Ala Gly Phe Gln Gly Val Arg Pro	
20 25 30	
Arg Ser Pro Ala Asp Ala Pro Leu Gly Met Arg Thr Thr Gly Ala Ser	
35 40 45	
Ala Ala Pro Lys Gln Gln Ser Arg Lys Ala His Arg Gly Thr Arg Arg	
50 55 60	
Cys Leu Ser Met Val Val Arg Ala Thr Gly Ser Ala Gly Met Asn Leu	
65 70 75 80	
Val Phe Val Gly Ala Glu Met Ala Pro Trp Ser Lys Thr Gly Gly Leu	
85 90 95	
Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Met Ala Ala Asn Gly His	
100 105 110	
Arg Val Met Val Ile Ser Pro Arg Tyr Asp Gln Tyr Lys Asp Ala Trp	
115 120 125	
Asp Thr Ser Val Val Ser Glu Ile Lys Val Ala Asp Glu Tyr Glu Arg	
130 135 140	
Val Arg Tyr Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe Val	
145 150 155 160	
Asp His Pro Cys Phe Leu Glu Lys Val Arg Gly Lys Thr Lys Glu Lys	
165 170 175	
Ile Tyr Gly Pro Asp Ala Gly Thr Asp Tyr Glu Asp Asn Gln Leu Arg	
180 185 190	
Phe Ser Leu Leu Cys Gln Ala Ala Leu Glu Ala Pro Arg Ile Leu Asp	
195 200 205	
Leu Asn Asn Asn Pro Tyr Phe Ser Gly Pro Tyr Gly Glu Asp Val Val	
210 215 220	
Phe Val Cys Asn Asp Trp His Thr Gly Leu Leu Ala Cys Tyr Leu Lys	
225 230 235 240	
Ser Asn Tyr Gln Ser Ser Gly Ile Tyr Arg Thr Ala Lys Val Ala Phe	
245 250 255	
Cys Ile His Asn Ile Ser Tyr Gln Gly Arg Phe Ser Phe Asp Asp Phe	
260 265 270	
Ala Gln Leu Asn Leu Pro Asp Arg Phe Lys Ser Ser Phe Asp Phe Ile	
275 280 285	
Asp Gly Tyr Asp Lys Pro Val Glu Gly Arg Lys Ile Asn Trp Met Lys	
290 295 300	
Ala Gly Ile Leu Gln Ala Asp Lys Val Leu Thr Val Ser Pro Tyr Tyr	
305 310 315 320	

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Ala Glu Glu Leu Ile Ser Gly Glu Ala Arg Gly Cys Glu Leu Asp Asn
 325 330 335

Ile Met Arg Leu Thr Gly Ile Thr Gly Ile Val Asn Gly Met Asp Val
 340 345 350

Ser Glu Trp Asp Pro Ala Lys Asp Lys Phe Leu Ala Ala Asn Tyr Asp
 355 360 365

Val Thr Thr Ala Leu Glu Gly Lys Ala Leu Asn Lys Glu Ala Leu Gln
 370 375 380

Ala Glu Val Gly Leu Pro Val Asp Arg Lys Val Pro Leu Val Ala Phe
 385 390 395 400

Ile Gly Arg Leu Glu Glu Gln Lys Gly Pro Asp Val Met Ile Ala Ala
 405 410 415

Ile Pro Glu Ile Leu Lys Glu Glu Asp Val Gln Ile Val Leu Leu Gly
 420 425 430

Thr Gly Lys Lys Lys Phe Glu Arg Leu Leu Lys Ser Val Glu Glu Lys
 435 440 445

Phe Pro Ser Lys Val Arg Ala Val Val Arg Phe Asn Ala Pro Leu Ala
 450 455 460

His Gln Met Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg Phe
 465 470 475 480

Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr Pro
 485 490 495

Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr Ile Met Glu Gly
 500 505 510

Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp Cys Asn Val Val
 515 520 525

Glu Pro Ala Asp Val Lys Lys Val Val Thr Thr Leu Lys Arg Ala Val
 530 535 540

Lys Val Val Gly Thr Pro Ala Tyr His Glu Met Val Lys Asn Cys Met
 545 550 555 560

Ile Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn Trp Glu Asp Val
 565 570 575

Leu Leu Glu Leu Gly Val Glu Gly Ser Glu Pro Gly Val Ile Gly Glu
 580 585 590

Glu Ile Ala Pro Leu Ala Met Glu Asn Val Ala Ala Pro
 595 600 605

<210> SEQ ID NO 11
 <211> LENGTH: 1818
 <212> TYPE: DNA
 <213> ORGANISM: Zea mays
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1815)

<400> SEQUENCE: 11

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1 5 10 15	
ggc gtc ccg gac gcg tcc acg ttc cgc cgc ggc gcc gcg cag ggc ctg	96
Gly Val Pro Asp Ala Ser Thr Phe Arg Gly Ala Ala Gln Gly Leu	
20 25 30	
agg ggg gcc cgg gcg tcg gcg gcg gcg gac acg ctc agc atg cgg acc	144
Arg Gly Ala Arg Ala Ser Ala Ala Ala Asp Thr Leu Ser Met Arg Thr	
35 40 45	
agc gcg cgc gcg gcg ccc agg cac cag cag cag gcg cgc cgc ggg ggc	192
Ser Ala Arg Ala Ala Pro Arg His Gln Gln Gln Ala Arg Arg Gly Gly	
50 55 60	

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agg ttc ccg tcg ctc gtc gtg tgc gcc agc gcc ggc atg aac gtc gtc	240
Arg Phe Pro Ser Leu Val Val Cys Ala Ser Ala Gly Met Asn Val Val	
65 70 75 80	
ttc gtc ggc gcc gag atg gcg ccg tgg agc aag acc ggc ggc ctc ggc	288
Phe Val Gly Ala Glu Met Ala Pro Trp Ser Lys Thr Gly Gly Leu Gly	
85 90 95	
gac gtc ctc ggc ggc ctg ccg ccg gcc atg gcc gcg aac ggg cac cgt	336
Asp Val Leu Gly Gly Leu Pro Pro Ala Met Ala Ala Asn Gly His Arg	
100 105 110	
gtc atg gtc gtc tct ccc cgc tac gac cag tac aag gac gcc tgg gac	384
Val Met Val Val Ser Pro Arg Tyr Asp Gln Tyr Lys Asp Ala Trp Asp	
115 120 125	
acc agc gtc gtg tcc gag atc aag atg gga gac ggg tac gag acg gtc	432
Thr Ser Val Val Ser Glu Ile Lys Met Gly Asp Gly Tyr Glu Thr Val	
130 135 140	
agg ttc ttc cac tgc tac aag cgc gga gtg gac cgc gtg ttc gtt gac	480
Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg Val Phe Val Asp	
145 150 155 160	
cac cca ctg ttc ctg gag agg gtt tgg gga aag acc gag gag aag atc	528
His Pro Leu Phe Leu Glu Arg Val Trp Gly Lys Thr Glu Glu Lys Ile	
165 170 175	
tac ggg cct gtc gct gga acg gac tac agg gac aac cag ctg cgg ttc	576
Tyr Gly Pro Val Ala Gly Thr Asp Tyr Arg Asp Asn Gln Leu Arg Phe	
180 185 190	
agc ctg cta tgc cag gca gca ctt gaa gct cca agg atc ctg agc ctc	624
Ser Leu Leu Cys Gln Ala Ala Leu Glu Ala Pro Arg Ile Leu Ser Leu	
195 200 205	
aac aac aac cca tac ttc tcc gga cca tac ggg gag gac gtc gtg ttc	672
Asn Asn Asn Pro Tyr Phe Ser Gly Pro Tyr Gly Glu Asp Val Val Phe	
210 215 220	
gtc tgc aac gac tgg cac acc ggc cct ctc tcg tgc tac ctc aag agc	720
Val Cys Asn Asp Trp His Thr Gly Pro Leu Ser Cys Tyr Leu Lys Ser	
225 230 235 240	
aac tac cag tcc cac ggc atc tac agg gac gca aag acc gct ttc tgc	768
Asn Tyr Gln Ser His Gly Ile Tyr Arg Asp Ala Lys Thr Ala Phe Cys	
245 250 255	
atc cac aac atc tcc tac cag ggc cgg ttc gcc ttc tcc gac tac ccg	816
Ile His Asn Ile Ser Tyr Gln Gly Arg Phe Ala Phe Ser Asp Tyr Pro	
260 265 270	
gag ctg aac ctc ccg gag aga ttc aag tcg tcc ttc gat ttc atc gac	864
Glu Leu Asn Leu Pro Glu Arg Phe Lys Ser Ser Phe Asp Phe Ile Asp	
275 280 285	
ggc tac gag aag ccc gtg gaa ggc cgg aag atc aac tgg atg aag gcc	912
Gly Tyr Glu Lys Pro Val Glu Gly Arg Lys Ile Asn Trp Met Lys Ala	
290 295 300	
ggg atc ctc gag gcc gac agg gtc ctc acc gtc agc ccc tac tac gcc	960
Gly Ile Leu Glu Ala Asp Arg Val Leu Thr Val Ser Pro Tyr Tyr Ala	
305 310 315 320	
gag gag ctc atc tcc ggc atc gcc agg ggc tgc gag ctc gac aac atc	1008
Glu Glu Leu Ile Ser Gly Ile Ala Arg Gly Cys Glu Leu Asp Asn Ile	
325 330 335	
atg cgc ctc acc ggc atc acc ggc atc gtc aac ggc atg gac gtc agc	1056
Met Arg Leu Thr Gly Ile Thr Gly Ile Val Asn Gly Met Asp Val Ser	
340 345 350	
gag tgg gac ccc agc agg gac aag tac atc gcc gtg aag tac gac gtg	1104
Glu Trp Asp Pro Ser Arg Asp Lys Tyr Ile Ala Val Lys Tyr Asp Val	
355 360 365	
tcg acg gcc gtg gag gcc aag gcg ctg aac aag gag gcg ctg cag gcg	1152
Ser Thr Ala Val Glu Ala Lys Ala Leu Asn Lys Glu Ala Leu Gln Ala	
370 375 380	

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gag gtc ggg ctc ccg gtg gac cgg aac atc ccg ctg gtg gcg ttc atc	1200
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ggc agg ctg gaa gag cag aag ggc ccc gac gtc atg gcg gcc gcc atc	1248
Gly Arg Leu Glu Glu Gln Lys Gly Pro Asp Val Met Ala Ala Ala Ile	
405 410 415	
ccg cag ctc atg gag atg gtg gag gac gtg cag atc gtt ctg ctg ggc	1296
Pro Gln Leu Met Glu Met Val Glu Asp Val Gln Ile Val Leu Leu Gly	
420 425 430	
acg ggc aag aag aag ttc gag cgc atg ctc atg agc gcc gag gag aag	1344
Thr Gly Lys Lys Lys Phe Glu Arg Met Leu Met Ser Ala Glu Glu Lys	
435 440 445	
ttc cca ggc aag gtg cgc gcc gtg gtc aag ttc aac gcg gcg ctg gcg	1392
Phe Pro Gly Lys Val Arg Ala Val Val Lys Phe Asn Ala Ala Leu Ala	
450 455 460	
cac cac atc atg gcc ggc gcc gac gtg ctc gcc gtc acc agc cgc ttc	1440
His His Ile Met Ala Gly Ala Asp Val Leu Ala Val Thr Ser Arg Phe	
465 470 475 480	
gag ccc tgc ggc ctc atc cag ctg cag ggg atg cga tac gga acg ccc	1488
Glu Pro Cys Gly Leu Ile Gln Leu Gln Gly Met Arg Tyr Gly Thr Pro	
485 490 495	
tgc gcc tgc gcg tcc acc ggt gga ctc gtc gac acc atc atc gaa ggc	1536
Cys Ala Cys Ala Ser Thr Gly Gly Leu Val Asp Thr Ile Ile Glu Gly	
500 505 510	
aag acc ggg ttc cac atg ggc cgc ctc agc gtc gac tgt aac gtc gtg	1584
Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp Cys Asn Val Val	
515 520 525	
gag ccg gcg gac gtc aag aag gtg gcc acc aca ttg cag cgc gcc atc	1632
Glu Pro Ala Asp Val Lys Lys Val Ala Thr Thr Leu Gln Arg Ala Ile	
530 535 540	
aag gtg gtc ggc acg ccg gcg tac gag gag atg gtg agg aac tgc atg	1680
Lys Val Val Gly Thr Pro Ala Tyr Glu Glu Met Val Arg Asn Cys Met	
545 550 555 560	
atc cag gat ctc tcc tgg aag ggc cct gcc aag aac tgg gag aac gtg	1728
Ile Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn Trp Glu Asn Val	
565 570 575	
ctg ctc agc ctc ggg gtc gcc ggc ggc gag cca ggg gtc gaa ggc gag	1776
Leu Leu Ser Leu Gly Val Ala Gly Gly Glu Pro Gly Val Glu Gly Glu	
580 585 590	
gag atc gcg ccg ctc gcc aag gag aac gtg gcc gcg ccc tga	1818
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595 600 605	

<210> SEQ ID NO 12

<211> LENGTH: 605

<212> TYPE: PRT

<213> ORGANISM: Zea mays

<400> SEQUENCE: 12

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Gly Val Pro Asp Ala Ser Thr Phe Arg Arg Gly Ala Ala Gln Gly Leu	20	25	30	
Arg Gly Ala Arg Ala Ser Ala Ala Ala Asp Thr Leu Ser Met Arg Thr	35	40	45	
Ser Ala Arg Ala Ala Pro Arg His Gln Gln Gln Ala Arg Arg Gly Gly	50	55	60	
Arg Phe Pro Ser Leu Val Val Cys Ala Ser Ala Gly Met Asn Val Val	65	70	75	80

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Phe	Val	Gly	Ala	Glu	Met	Ala	Pro	Trp	Ser	Lys	Thr	Gly	Gly	Leu	Gly	85	90	95	
Asp	Val	Leu	Gly	Gly	Leu	Pro	Pro	Ala	Met	Ala	Ala	Asn	Gly	His	Arg	100	105	110	
Val	Met	Val	Val	Ser	Pro	Arg	Tyr	Asp	Gln	Tyr	Lys	Asp	Ala	Trp	Asp	115	120	125	
Thr	Ser	Val	Val	Ser	Glu	Ile	Lys	Met	Gly	Asp	Gly	Tyr	Glu	Thr	Val	130	135	140	
Arg	Phe	Phe	His	Cys	Tyr	Lys	Arg	Gly	Val	Asp	Arg	Val	Phe	Val	Asp	145	150	155	160
His	Pro	Leu	Phe	Leu	Glu	Arg	Val	Trp	Gly	Lys	Thr	Glu	Glu	Lys	Ile	165	170	175	
Tyr	Gly	Pro	Val	Ala	Gly	Thr	Asp	Tyr	Arg	Asp	Asn	Gln	Leu	Arg	Phe	180	185	190	
Ser	Leu	Leu	Cys	Gln	Ala	Ala	Leu	Glu	Ala	Pro	Arg	Ile	Leu	Ser	Leu	195	200	205	
Asn	Asn	Asn	Pro	Tyr	Phe	Ser	Gly	Pro	Tyr	Gly	Glu	Asp	Val	Val	Phe	210	215	220	
Val	Cys	Asn	Asp	Trp	His	Thr	Gly	Pro	Leu	Ser	Cys	Tyr	Leu	Lys	Ser	225	230	235	240
Asn	Tyr	Gln	Ser	His	Gly	Ile	Tyr	Arg	Asp	Ala	Lys	Thr	Ala	Phe	Cys	245	250	255	
Ile	His	Asn	Ile	Ser	Tyr	Gln	Gly	Arg	Phe	Ala	Phe	Ser	Asp	Tyr	Pro	260	265	270	
Glu	Leu	Asn	Leu	Pro	Glu	Arg	Phe	Lys	Ser	Ser	Phe	Asp	Phe	Ile	Asp	275	280	285	
Gly	Tyr	Glu	Lys	Pro	Val	Glu	Gly	Arg	Lys	Ile	Asn	Trp	Met	Lys	Ala	290	295	300	
Gly	Ile	Leu	Glu	Ala	Asp	Arg	Val	Leu	Thr	Val	Ser	Pro	Tyr	Tyr	Ala	305	310	315	320
Glu	Glu	Leu	Ile	Ser	Gly	Ile	Ala	Arg	Gly	Cys	Glu	Leu	Asp	Asn	Ile	325	330	335	
Met	Arg	Leu	Thr	Gly	Ile	Thr	Gly	Ile	Val	Asn	Gly	Met	Asp	Val	Ser	340	345	350	
Glu	Trp	Asp	Pro	Ser	Arg	Asp	Lys	Tyr	Ile	Ala	Val	Lys	Tyr	Asp	Val	355	360	365	
Ser	Thr	Ala	Val	Glu	Ala	Lys	Ala	Leu	Asn	Lys	Glu	Ala	Leu	Gln	Ala	370	375	380	
Glu	Val	Gly	Leu	Pro	Val	Asp	Arg	Asn	Ile	Pro	Leu	Val	Ala	Phe	Ile	385	390	395	400
Gly	Arg	Leu	Glu	Glu	Gln	Lys	Gly	Pro	Asp	Val	Met	Ala	Ala	Ala	Ile	405	410	415	
Pro	Gln	Leu	Met	Glu	Met	Val	Glu	Asp	Val	Gln	Ile	Val	Leu	Leu	Gly	420	425	430	
Thr	Gly	Lys	Lys	Lys	Phe	Glu	Arg	Met	Leu	Met	Ser	Ala	Glu	Glu	Lys	435	440	445	
Phe	Pro	Gly	Lys	Val	Arg	Ala	Val	Val	Lys	Phe	Asn	Ala	Ala	Leu	Ala	450	455	460	
His	His	Ile	Met	Ala	Gly	Ala	Asp	Val	Leu	Ala	Val	Thr	Ser	Arg	Phe	465	470	475	480
Glu	Pro	Cys	Gly	Leu	Ile	Gln	Leu	Gln	Gly	Met	Arg	Tyr	Gly	Thr	Pro	485	490	495	
Cys	Ala	Cys	Ala	Ser	Thr	Gly	Gly	Leu	Val	Asp	Thr	Ile	Ile	Glu	Gly	500	505	510	

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Lys Thr Gly Phe His Met Gly Arg Leu Ser Val Asp Cys Asn Val Val
515 520 525

Glu Pro Ala Asp Val Lys Lys Val Ala Thr Thr Leu Gln Arg Ala Ile
530 535 540

Lys Val Val Gly Thr Pro Ala Tyr Glu Glu Met Val Arg Asn Cys Met
545 550 555 560

Ile Gln Asp Leu Ser Trp Lys Gly Pro Ala Lys Asn Trp Glu Asn Val
565 570 575

Leu Leu Ser Leu Gly Val Ala Gly Gly Glu Pro Gly Val Glu Gly Glu
580 585 590

Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Ala Pro
595 600 605

We claim:

1. A genetically modified monocotyledonous plant cell that comprises a waxy mutation that results in the synthesis of a starch with an apparent amylose content of less than 5% by weight and that comprises a foreign nucleic acid molecule that increases the expression of a starch synthase II, and a foreign nucleic acid molecule that increases the expression of a glucan water dikinase, each compared to a corresponding monocotyledonous plant cell that does not comprise said foreign nucleic acid molecules, wherein said starch has a hot-swelling power of between 60 to 100 g/g.

2. A monocotyledonous plant comprising the genetically modified plant cell of claim 1.

3. The monocotyledonous plant of claim 2, wherein said plant is rice, maize or wheat.

4. A propagation material of monocotyledonous plants, wherein said propagation material comprises the genetically modified plant cell of claim 1.

5. A method of generating a genetically modified monocotyledonous plant, said method comprising the following steps:

a) genetically modifying a monocotyledonous plant cell comprising the following steps:

i) introducing, into the plant cell, a foreign nucleic acid molecule that increases the expression of a starch synthase II in comparison with a corresponding wild-type plant cell that does not comprise said foreign nucleic acid molecule,

ii) introducing, into the plant cell, a foreign nucleic acid molecule that increases the expression of a glucan water dikinase in comparison with a corresponding wild-type plant cell that does not comprise said foreign nucleic acid molecule,

iii) introducing, into the plant cell, a foreign nucleic acid molecule that reduces the expression of a GBSSI in comparison with a corresponding wild-type plant cells that does not comprise said foreign nucleic acid molecule,

where steps i to iii can be carried out in any sequence, individually or simultaneously; and

b) regenerating a plant from the plant cell of step a).

6. A process for the preparation of a modified starch, said process comprising the step of extracting the starch from the genetically modified plant cell of claim 1.

7. A process for the preparation of flours, said process comprising the step of grinding parts of the plant of claim 2.

8. The method of claim 5, further comprising the following steps:

c) generating further plants by isolating plant cells from a plant of step b) and repeating steps a) and b); and

d) repeating step c) until a plant has been generated which has an increased expression of a starch synthase II, an increased expression of a glucan water dikinase, and a reduced expression of a GBSSI, in comparison with a corresponding wild-type plant that has not been genetically modified by introducing the foreign nucleic acid molecules of claim 5.

9. A process for the preparation of flours, said process comprising the step of grinding parts of the propagation material of claim 4.

10. A process for the preparation of flours, said process comprising the step of grinding parts of the plants produced by the method of claim 5.

11. The genetically modified monocotyledonous plant cell of claim 1, wherein the plant cell comprises

at least one foreign nucleic acid molecule encoding a starch synthase II; and

at least one foreign nucleic acid molecule encoding a glucan water dikinase.

12. The genetically modified monocotyledonous plant cell of claim 11, wherein the at least one foreign nucleic acid molecule encoding a starch synthase II comprises:

i) a nucleic acid molecule encoding a protein comprising the amino acid sequence of SEQ. ID. NOs.: 4 or 6;

ii) a nucleic acid molecule comprising the nucleic acid sequence of SEQ. ID. NOs.: 3 or 5; or

iii) a nucleic acid molecule that hybridizes under stringent conditions with at least one strand of the nucleic acid molecule described in ii), wherein the stringent conditions are:

hybridization buffer: 2×SSC, 10×Denhardt solution (Ficoll 400+PEG+BSA; ratio 1:1:1), 0.1% SDS; 5 mM EDTA, 50 mM Na₂HPO₄, 250 μg/ml of herring sperm DNA, 50 μg/ml of tRNA, or 25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS;

hybridization temperature: T=65 to 68° C.;

wash buffer: 0.1×SSC; 0.1% SDS; and

wash temperature: T=65 to 68° C.

13. The genetically modified monocotyledonous plant cell of claim 11, wherein the at least one foreign nucleic acid molecule encoding a glucan water dikinase comprises:

i) a nucleic acid molecule encoding a protein comprising the amino acid sequence of SEQ. ID. NO.: 2;

ii) a nucleic acid molecule comprising the nucleic acid sequence of SEQ. ID. NO.: 1; or

111

iii) a nucleic acid molecule that hybridizes under stringent conditions with at least one strand of the nucleic acid molecule described in ii), wherein the stringent conditions are:

hybridization buffer: 2×SSC, 10×Denhardt solution (Ficoll 400+PEG+BSA; ratio 1:1:1), 0.1% SDS; 5 mM EDTA, 50 mM Na₂HPO₄, 250 μg/ml of herring sperm DNA, 50 μg/ml of tRNA, or 25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS;

hybridization temperature: T=65 to 68° C.;

wash buffer: 0.1×SSC; 0.1% SDS; and

wash temperature: T=65 to 68° C.

14. A genetically modified monocotyledonous plant cell comprising a foreign nucleic acid molecule that reduces the expression of an endogenous GBSSI, a foreign nucleic acid molecule that increases expression of a starch synthase II, and a foreign nucleic acid molecule that increases expression of a glucan water dikinase, each compared to a corresponding monocotyledonous plant cell that does not comprise said foreign nucleic acid molecules, wherein said plant cell synthesizes a starch having a hot-swelling power of between 60 to 100 g/g.

15. The genetically modified monocotyledonous plant cell of claim 14, wherein the foreign nucleic acid molecule that reduces the expression of an endogenous GBSSI comprises:

i) a nucleic acid molecule encoding a protein comprising the amino acid sequence of SEQ. ID. NO.: 8, 10, or 12;

ii) a nucleic acid molecule comprising the nucleic acid sequence of SEQ. ID. NO.: 7, 9, or 11; or

iii) a nucleic acid molecule that hybridizes under stringent conditions with at least one strand of the nucleic acid molecule described in ii), wherein the stringent conditions are:

hybridization buffer: 2×SSC, 10×Denhardt solution (Ficoll 400+PEG+BSA; ratio 1:1:1), 0.1% SDS; 5 mM EDTA, 50 mM Na₂HPO₄, 250 μg/ml of herring sperm DNA, 50 μg/ml of tRNA, or 25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS;

112

hybridization temperature: T=65 to 68° C.;

wash buffer: 0.1×SSC; 0.1% SDS; and

wash temperature: T=65 to 68° C.

16. The genetically modified monocotyledonous plant cell of claim 14, wherein the foreign nucleic acid molecule that reduces the expression of an endogenous GBSSI comprises

(i) a nucleic acid molecule encoding at least one antisense RNA that reduces the expression of at least one endogenous gene encoding a GBSSI protein;

(ii) a nucleic acid molecule which, via a co-suppression effect, reduces the expression of at least one endogenous gene encoding a GBSSI protein; or

(iii) a nucleic acid molecule that simultaneously encodes at least one antisense RNA and at least one sense RNA, where said antisense RNA and said sense RNA form a double-stranded RNA molecule that reduces the expression of at least one endogenous gene encoding a GBSSI protein.

17. A method of generating a genetically modified monocotyledonous plant comprising the following steps:

a) genetically modifying a monocotyledonous plant cell that comprises a waxy mutation that results in the synthesis of a starch having an apparent amylose content of less than 5% comprising the following steps:

i) introducing, into the plant cell, a foreign nucleic acid molecule that increases the expression of a starch synthase II in comparison with a corresponding wild-type plant cell that does not comprise said foreign nucleic acid molecule, and

ii) introducing, into the plant cell, a foreign nucleic acid molecule that increases the expression of a glucan, water dikinase in comparison with a corresponding wild-type plant cell that does not comprise said foreign nucleic acid molecule

where steps i to ii can be carried out in any sequence, individually or simultaneously; and

b) regenerating a plant from the plant cell of step a).

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